Department of the Army
Pamphlet 385–69

Safety

Biological Defense Safety Program

Headquarters
Department of the Army
Washington, DC
31 December 1993

Unclassified

SUMMARY of CHANGE

DA PAM 385–69
Biological Defense Safety Program

This pamphlet--

- Prescribes objectives for the safety program (para 1-4).
- Contains requirements for maintaining safe laboratory operations and monitoring the health of laboratory personnel (chap 2).
- Contains requirements for training all personnel in the safety program (para 3-2).
- Contains requirements for operating laboratories at each biosafety level (chap 3).
- Contains requirements for planning a response to any emergency (para 3-9).
- Contains requirements for using personal protective equipment (chap 4).
- Contains requirements for decontaminating and disposing of infectious materials and contaminated equipment (chap 5).
- Contains requirements for importing, shipping, and transporting etiologic agents (chap 6).
- Contains requirements for designing and maintaining facilities with safety features equal to the possible risks (chap 7).
- Contains requirements for engineering controls to promote safe operations (chap 8).

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Biological Defense Safety Program

By Order of the Secretary of the Army:

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General, United States Army
Chief of Staff

Official:

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History. This UPDATE printing provides a new Department of the Army pamphlet.

Summary. This pamphlet establishes the Army safety program for all aspects of the Biological Defense Program. It provides new Department of the Army policy on the management of the Biological Defense Safety Program. This pamphlet implements the Centers for Disease Control — National Institutes of Health Guidelines on Laboratory Biosafety, Department of Defense and Department of the Army policy statements, and other Federal regulations. It prescribes procedures for safety studies and reviews of biological defense research, development, test and evaluation projects, and prescribes safety precautions and procedures applicable to contractor operations.

Applicability. This pamphlet applies to Active Army, Army National Guard, U.S. Army Reserve, Army civilian employees, Army contractors with a responsibility for biological defense research, development, test, and evaluation operations, and other Federal agencies engaged in biological defense operations for the Department of the Army.

Proponent and exception authority. The proponent of this pamphlet is the Director of the Army Staff (DAS). The DAS has the authority to approve exceptions to this pamphlet that are consistent with controlling law and regulation.The DAS may delegate this approval authority, in writing, to a division chief under their supervision within the proponent agency who holds the grade of colonel or the civilian equivalent.

Interim changes. Interim changes to this pamphlet are not official unless they are authenticated by the Administrative Assistant to the Secretary of the Army. Users will destroy interim changes on their expiration dates unless sooner superseded or rescinded.

Suggested Improvements. Users are invited to send comments and suggested improvements on DA Form 2028 (Recommended Changes to Publications and Blank Forms) directly to Army Safety Office (DACS–SF), Chief of Staff, 200 Army Pentagon, Washington DC 20310–0200.

Distribution. Distribution of this publication is made in accordance with the special distribution list provided by the proponent, intended for command level D for Active Army, Army National Guard, and U.S.Army Reserve.

Contents (Listed by paragraph and page number)

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Contents—Continued

Chapter 1
Introduction

1–1. Purpose
This pamphlet prescribes the technical safety requirements for the use, handling, shipment, storage, and disposal of etiologic agents used in research, development, test, and evaluation (RDTE)for the Biological Defense Program (BDP). The requirements stated in this pamphlet apply to all elements of the Army to include the Army National Guard (ARNG) and the U.S. Army Reserve (USAR) and its contractors and subcontractors who use, produce, store, handle, or ship etiologic agents in support of the BDP, regardless of the source of the agents.

1–2. References
Required and related publications and prescribed and referenced forms are listed in appendix A.

1–3. Explanation of abbreviations and terms
Abbreviations and special terms used in this pamphlet are explained in the glossary.

1–4. Background
The U.S. Army BDP, on behalf of the Department of Defense, supports research, development, test, and evaluation (RDTE) efforts to maintain and develop defensive measures and materiel to meet potential biological warfare threats. The program's objectives are to develop measures for identification, detection, treatment, protection against, and decontamination of these threats. To meet the program objectives, etiologic agents are used to conduct the necessary RDTE. This pamphlet contains information on the safe use, handling, storage, shipment, and disposal of etiologic agents. This pamphlet describes requirements based on the Centers for Disease Control-National Institute of Health (CDC) (NIH) guidelines, Biosafety in Microbiological and Biomedical Laboratories, and establishes guidelines for toxins.

Chapter 2
Administration

2–1. Safety administration
Each BDP institution must have a safety program that complies with AR 385–10, AR 385–69, and this pamphlet.In addition, the safety program must be designed to ensure compliance with—

a. Occupational Safety and Health Administration (OSHA)requirements for health and safety.

b. Environmental Protection Agency (EPA) regulations designed to implement the Resource Conservation and Recovery Act (RCRA) and the National Environmental Policy Act (NEPA).

c. Nuclear Regulatory Commission (NRC) requirements for safe handling of radioactive isotopes (when applicable).

d. NIH Guidelines for Research Involving Recombinant Deoxyribonucleic Acid (DNA) Molecules.

e. Relevant national, State, and local regulations.

f. Any requirements of applicable accrediting bodies.

2–2. Goals of a laboratory safety program
The goals of the laboratory safety program are to protect those working in the laboratory, others who may potentially be exposed to hazards in the laboratory, and the environment. In addition, a laboratory safety program should ensure that hazardous materials will be handled and disposed of in such a way that people, other living organisms, and the environment are protected from harm.Safety awareness must be a part of everyone's habits, and can only be achieved if all senior and responsible staff have a sincere, visible, and continuing interest in preventing injuries and occupational illnesses. Laboratory personnel, for their part, must carry out their work in a way that protects themselves and their fellow workers.

a. Laboratory safety. The safety program will be carried out as stated in AR 385–69. Additionally, the program will contain the following:

(1) A commander or institute director, along with all personnel, having a continuing, observable, and known commitment to the safety program.

(2) An effective institutional safety program requiring a safety officer appropriately trained in relevant safety technology.This individual, besides supplying advice and recommendations, will ensure that records are kept showing that the institution's physical facilities and safety rules are internally consistent and compatible with potential risks, as well as in compliance with all applicable laws, regulations, and guidelines.

(3) A commander who ensures safety in every department or other equivalent administrative unit of the institution. Ensuring safe operations is an integral function of each level of management through the first line supervisor. The safety office staff must work closely with administrators and investigators to develop and implement written policies and practices that promote safe laboratory work. Collectively, this group routinely must monitor current operations and practices, see that appropriate audits are maintained, and continue to seek ways to improve the safety program.

(4) Guidelines enforcing safety as a critical job element for each member of the scientific and technical staff. Each individual working in the laboratory must perform his or her job in a manner consistent with safety policy and training.

(5) Guidelines that require that if laboratory goals dictate operations or substances not suited to the existing facilities or equipment, the laboratory supervisor will, assisted by the safety officer, advise and assist the laboratory worker in developing or obtaining adequate facilities or equipment and designing appropriate work procedures.

(6) A supervisor authorizing each specific operation, delineate appropriate safety procedures, and instructing those who will carry out the operation.

(7) Identification of potential hazards before work with etiologic agents begins, and implementation of actions necessary to avoid accidents and illnesses. This practice, called a job safety analysis, consists of breaking a job down into its logical steps, analyzing each for its hazard potential, and deciding the safe procedures to use. The process will be designed by a project director with input from employees, and each step with potential for exposure or other incidents must be described in writing in a standing operating procedure (SOP). All such SOPs will be approved by, at a minimum, the commander or institute director and the safety officer.

(8) A job safety analysis that includes a consideration of health hazards identified in AR 40–10 and of maximum credible events as described in AR 385–69, paragraph 2–8.

b. Safety plans. Clearly defined, published safety rules and monitoring procedures for compliance must be established. These rules will be readily available, in writing, for all involved in laboratory operations. This goal may be accomplished by preparing or modifying a facility safety plan, laboratory safety manual, safety and occupational health program document, or equivalent. This plan will—

(1) Be coordinated with institutional and Federal, State, and local emergency services.

(2) Be practiced with the emergency groups whose services are part of that plan prior to any need for their services, so that they can become familiar with any potential problem areas that may be encountered when they are called upon for assistance.

(3) Describe the method of rapid communication (for example, telephone, alarms, and so forth) that will be used during an emergency.

(4) Describe the institution's etiologic agent labeling system.

(5) Describe the institution's requirements for testing engineering controls (for example, biological safety cabinets and high efficiency particulate air (HEPA) filters) and essential safety equipment (for example, autoclaves) that are used to conduct RDTE funded by the BDP.

(6) Be used to appoint and train personnel responsible for handling an emergency.

(7) Require that emergency telephone numbers be posted, so that

emergency service personnel know whom to contact at all times of the day or night.

(8) Describe the institution's rules that have been established and are practiced to limit access to the facilities where etiologic agents under the sponsorship of the BDP are handled. The rules will include the following requirements:

(a) Access to biosafety level (BL)–1 and BL–1 large-scale (LS) laboratories is limited or restricted at the discretion of the commander or institute director when experiments are in progress.

(b) Access to areas classified as BL–2, BL–2 LS, or where work with toxins is conducted, is limited by the commander or institute director when work with etiologic agents is in progress.Individuals who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory. Only persons who have been advised of the potential hazard and meet any specific entry requirements (for example, immunization) may enter the individual laboratory or animal rooms.The commander or institute director must assess each circumstance and determine who may enter or work in the laboratory.

(c) Access to areas classified as BL–3 or BL–3 LS is limited as stated in (8)(b) above, and is restricted to those persons whose presence in the facility or individual laboratory rooms is required for program or support purposes. Individuals under 18 years of age may not enter the controlled area.

(d) Access to BL–4 facilities is limited as stated in(8)(b) and (c) above. This is done with secure, locked doors with access controlled by the commander or institute director, safety officer, or other person responsible for the physical security of the facility. Before entry, all persons will be advised as to the appropriate safeguards for ensuring their safety. Authorized persons must comply with these instructions and all other applicable entry and exit procedures. A logbook will be maintained for all personnel to indicate the date and time of each entry and exit. A card-key activated computer record (or other electronic entry device) may be used if it indicates the date and time of both entry and exit.

(9) Describe the system that is developed and is operational for the reporting of accidents and exposures, employee absenteeism, and for the medical surveillance of potential laboratory associated illnesses.

c. Safety meetings and safety committees. In effective safety programs, everyone associated with the laboratory becomes involved. This is done by ensuring maximum participation in planning and by conducting group safety meetings.

(1) A staff safety committee, consisting of the commander or institute director or his or her designated representative, research supervisors, managers, medical personnel, employees, and the safety officer, will be established. This group leads the safety effort, reviews mishaps, and recommends changes in policies, safety program, or equipment as needed to improve safety.

(2) Safety committees will meet at least quarterly and minutes will be prepared and maintained for at least 3 years.

(3) When work with recombinant DNA molecules is undertaken, an institutional biosafety committee (IBC) for review of such work will be established and will function as stated in the NIH Guidelines for Research Involving Recombinant DNA Molecules. (See app A.)

d. SOPs. Besides the documented safety program that will be in effect, each institution will require that an SOP be established for each unique biological defense RDTE operation. The SOPs will meet the criteria stated in AR 385–69 and be reviewed and updated annually. A copy of the SOP will be maintained in the work area. In addition, SOPs will address the following issues:

(1) The unique hazards introduced by the activity in the work area.

(2) The methods of controlling these hazards.

(3) Any unique procedures and requirements needed that are not described as universally required in the safety plan (for example, signs, waste disposal, immunizations, emergency procedures, and personnel monitoring).

(4) Specialized orientation or training of personnel beyond that required in the safety plan.

(5) Ways of ensuring that the unique procedures are followed.

(6) Emergency procedures.

e. Safety communications. Safety communications alert people to newly recognized hazards, remind them of basic biological safety principles, and instill positive attitudes toward safety. Training requirements are also found in paragraph 3–1 *b*. A system of communication will be established to—

(1) Implement a biological safety training program for all personnel working with hazardous biological or chemical materials.

(2) Publish information addressing useful biological safety advice and accounts of laboratory accidents, along with the lessons to be learned from them.

(3) Make reference books and regulations concerning laboratory hazards, occupational health, and proper laboratory practices readily available.

(4) Assure that material safety data sheets (MSDS) for hazardous chemicals used in the laboratory are readily available to all employees.

f. Safety audits. One of the essential elements of a good safety program is the conduct of periodic audits of the safety performance in a laboratory. Observing individual safety practices and checking the operability of safety equipment and compliance with safety rules must be part of the audit.

(1) An individual and an alternate will be appointed for each laboratory or room where BDP work is conducted. On a daily basis he or she will monitor the conduct of personnel within their rooms and maintenance of the room to see that they comply with the safety program and SOPs.

(2) Supervisors will ensure that their projects comply with applicable safety requirements and will audit their areas at least weekly to ensure compliance.

(3) The safety officer or his or her qualified designee will inspect the institution's BL–1, BL–2, and toxin laboratories quarterly. BL–3 and BL–4 laboratories and those in which dry forms of highly potent toxins are handled will be inspected monthly by safety and occupational health professionals.These inspections will be unannounced and include coverage of general safety practices as well as features specific to a particular biosafety level.

(a) Reports of deficiencies or procedures that create a potentially life threatening situation will be made directly to supervisory personnel and the commander or institute director and actions will be taken immediately to correct the situation. The operation will not continue until every deficiency is corrected.

(b) Reports of deficiencies for other than life-threatening situations will be made as soon as possible to the appropriate supervisor, with copies furnished to the commander or institute director. If a problem is widespread, all affected personnel will be notified.

(4) Supervisory personnel notified of safety deficiencies by the safety officer will ensure that the people directly concerned are contacted and that the deficiencies are remedied before operations are resumed.

(5) Malfunctioning equipment must be reported to the appropriate individuals, labeled to indicate that it should not be used, and repaired promptly.

(6) As a minimum, the audits conducted by the safety officer or his or her qualified designee will cover the items listed in appendix B.

g. Documentation. Records, documenting the following items, will be maintained for 3 years:

(1) Safety audits and the corrective measures.

(2) Risk assessments for proposed new laboratory procedures.

(3) Annual reviews of established SOPs.

(4) Training.

(5) Engineering controls and protective equipment certifications and tests.

(6) Safety committee meeting minutes and recommendations.

(7) Any outside auditor comments and responses.

2–3. Occupational health

An occupational health program will be implemented per AR 40–5, chapter 5, for all employees whose employment requires that they

conduct duties in a BDP etiologic agent area. Essential elements of the program will include—

a. Medical surveillance examinations. Medical examinations by a licensed medical doctor will be given prior to employment, at least every 3 years thereafter, and upon termination of duties requiring access to laboratories where etiologic agents are used. When full medical examinations are not given annually, health professionals will perform annual health screening. Safety and health professionals will ensure that medical examiners are made aware of all hazardous substances each employee works with at the time of the medical examination. The physician's findings will include assessment of whether an employee has any health condition that would preclude work with etiologic agents. If any of the findings obtained during the examination are outside the normal range, the employee's supervisor and the employee will be notified and counseled on the courses of action available. In addition, a safety and health audit will be conducted to identify any potential occupational causes for the abnormalities, and corrective measures will be taken if applicable.

b. Serum samples. When appropriate, considering the agents handled, baseline serum samples for laboratory and other at-risk personnel will be collected and stored for their biologically useful lifetime, but not longer than 40 years. Additional serum specimens will be collected periodically, based upon the agents handled, or as required by participation in a special immunizations program. SOPs will be written detailing the collection procedures and periods if serum sampling is deemed necessary.

c. Assignment of personnel. Personnel assigned duties in work areas where etiologic agents are used will be evaluated to determine their suitability for their assigned tasks by the institutional medical authority. Only personnel who are physically and mentally capable of working in biocontainment areas (BL–3 and BL–4) or with toxins will be assigned to these duties.

d. Immunization of at-risk personnel. The guidelines for immunizations in the latest edition of the American College of Physicians' Guide for Adult Immunizations and recommendations of Health and Human Services (HHS) in publication number (NIH) 88-8395 will be followed. A resource list for available immunizations for personnel at risk is given in Table 2–1.

e. Reporting exposures. Spills and mishaps which result in observable, known, or potential exposures to etiologic agents will be immediately reported to the supervisor, the safety officer, the responsible medical personnel, and the commander. Appropriate medical evaluation, surveillance, and treatment will be provided and written records of these occurrences will be maintained for 40 years. A Med-16 report will be initiated (see AR 40–400).

f. Quarantine. When etiologic agents designated as BL–4 are handled, a facility for the quarantine, isolation, and medical care of personnel with potential or known laboratory associated exposures will be available.

2–4. Medical records

Army activities will maintain medical records in accordance with AR 40–66 for all military and Department of the Army (DA)civilian employees who work with etiologic agents under sponsorship of the BDP.

Table 2–1
Resource list for immunoprophylaxis of personnel at risk

Description of Disease	Product	Recommended For Use In	Source of Product
Anthrax	Inactivated vaccine	Personnel working regularly with cultures, diagnostic materials, or infected animals.	USAMRIID [1]
Botulism	Pentavalent toxoid (A,B,C,D,E) (IND) [2]	Personnel working regularly with cultures or toxin.	CDC [3]
Cholera	Inactivated vaccine	Personnel working regularly with large volumes or high concentrations of infectious materials.	Commercially available
Diphtheria Tetanus (Adult)	Combined toxoid	All laboratory and animal care personnel irrespective of agents handled.	Commercially available
Eastern equine encephalitis (EEE)	Inactivated vaccine (IND) [2]	Personnel who work directly and regularly with EEE in the laboratory.	USAMRIID [1]
Hepatitis A	Immune serum globulin [ISG (Human)]	Animal care personnel working directly with chimpanzees naturally or experimentally infected with Hepatitis A virus.	Commercially available
Hepatitis B	Serum derived or recombinant vaccine	Personnel working regularly with human blood and blood components.	Commercially available
Influenza	Inactivated vaccine	(Vaccines prepared from earlier isolated strains may be of little value in personnel working with recent isolates from humans or animals.)	Commercially available
Japanese Encephalitis (JE)	Inactivated vaccine (IND) [2]	Personnel who work directly and regularly with JE virus in the laboratory.	CDC [3]
Measles	Live attenuated virus vaccine	Measles-susceptible personnel working with the agent or potentially infectious clinical materials.	Commercially available
Meningococcal Meningitis	Purified polysaccharide vaccine	Personnel working regularly with large volumes or high concentrations of infectious materials (does not protect against infection with group B meningococcus).	Commercially available
Plague	Inactivated vaccine	Personnel working regularly with cultures of Yersinia pestis or infected rodents or fleas	Commercially available
Poliomyelitis	Inactivated (IPV) and live attenuated (OPV) vaccines	Polio-susceptible personnel working with the virsus or entering laboratories or animal rooms where the virus is in use.	Commercially available

Table 2–1
Resource list for immunoprophylaxis of personnel at risk—Continued

Description of Disease	Product	Recommended For Use In	Source of Product
Pox viruses (Vaccinia, Cowpox, or Monkey Pox viruses)	Live (lyophilized) vaccinia virus	Personnel working with orthopox viruses transmissible to humans, with animals infected with these agents, and persons entering areas where these viruses are in use.	CDC [3]
Q Fever (Phase II) vaccine	Inactivated (IND) [2]	Personnel who have no demonstrable sensitivity to Q fever antigen and who are at high risk of exposure to infectious materials or animals.	USAMRIID [1]
Rabies	Human diploid line cell inactivated vaccine	Personnel working with all strains of rabies virus, with infected animals, or persons entering areas where these activities are conducted.	Commercially available
Rift Valley Fever	Inactivated virus vaccine (IND) [2]	All laboratory and animal care personnel working with the agent or infected animals and all personnel entering laboratories or animal rooms when the agent is in use.	USAMRIID [1]
Rubella	Live attenuated virus vaccine	Rubella-susceptible personnel, especially women, working with "wild" strains or in areas where these viruses are in use.	Commercially available
Tuberculosis	Live, attenuated (BCG) bacterial vaccine	BCG vaccine ordinarily is not used by laboratory personnel in the United States.	Commercially available
Tularemia	Live attenuated bacterial vaccine (IND) [2]	Personnel working regularly with cultures or infected animals or persons entering areas where the agent or infected animals are in use.	USAMRIID [1]
Typhoid	Inactivated vaccine	Personnel who have no demonstrated sensitivity to the vaccine and who work regularly with cultures.	Commercially available
Venezuelan equine (VEE) encephalitis	Live attenuated (TC83) viral vaccine (IND)[2]	Personnel working with VEE and the Equine Cabassou, Everglades, Mucambo, and Tonate viruses, or who enter areas where these viruses are in use.	USAMRIID [1]
Western equine encephalitis (WEE)	Inactivated vaccine (IND) [2]	Personnel who work directly and regularly with WEE virus in the laboratory.	USAMRIID [1]
Yellow Fever	Live attenuated (17D) virus vaccine	Personnel working with virulent and avirulent strains of Yellow Fever virus.	Commercially available

Legend for Table 2-1:
Source: Adapted from recommendations of the PHS Immunization Practices Advisory Committee and Biosafety in Microbiological and Biomedical Laboratories.
[1] For information, contact: U. S. Army Medical Materiel Development Activity, Fort Detrick, Frederick, MD 21702, telephone: (301) 663–7661.
[2] Investigational New Drug (IND)
[3] Clinical Medicine Branch, Division of Host Factors, Center for Infectious Disease, Centers for Disease Control, Atlanta, GA 3033, telephone: (404) 639–3356.

Chapter 3
Operational Requirements

3–1. Personnel prerequisites

a. Medical. Before assignment to work with etiologic agents, personnel will be evaluated by the appropriate medical personnel with respect to their assignments and will be evaluated in the medical surveillance program described in paragraph 2–3.

b. Training. All personnel directly or indirectly involved with containment or handling of known and potentially biohazardous material will receive instruction that adequately prepares them for their assigned duties. Training will be given by occupationally qualified personnel as determined by the commander or director. This training will be documented and will include—

(1) General training as follows:

(a) Personal hygiene related to laboratory work.

(b) Laboratory practices.

(c) Personal protective equipment.

(d) Effective use of engineering controls.

(e) Packaging, transportation, and shipment of etiologic agents(when applicable).

(f) Hazardous and infectious waste disposal, handling, and minimization procedures.

(g) Safety training program for all personnel working with hazardous biological or chemical materials.

(2) Training conducted specifically for the facilities that the individual will be working in, including—

(a) Procedures for the facility.

(b) Reporting incidents and accidents.

(c) Labeling and posting of signs.

(d) Biohazardous waste handling, approaches to minimizing the volume of waste, decontamination, packaging, and disposal.

(e) Emergency procedures.

(3) Additional general training required for work in facilities where viable etiologic agents are present.

(a) Aseptic technique and procedures to include hands-on instruction and demonstration of proficiency.

(b) Concept and definition of biosafety levels.

(c) Disinfection and sterilization.

(d) Safe use of workplace equipment, for example autoclaves and centrifuges.

(e) Monitoring and auditing requirements.

(f) Precautions for handling blood, tissues, and body fluids(when applicable).

(g) The infectivity, pathogenicity, modes of transmission, and medical surveillance requirements of specific agents.

(h) Training for all new employees will include a period of supervised orientation in the facilities by a scientist or technician with specific training in the procedures and properties of the etiologic agents in use. During the training period, new laboratory personnel

will be under the constant supervision of appropriately trained personnel.

(i) Personnel who are assigned tasks in BL–2, BL–3, or BL–4 facilities will also have specific training in handling pathogens.

(j) Personnel assigned duties in a BL–4 facility will also have specific and thorough training in handling extremely hazardous infectious agents, the primary and secondary containment functions of standard and special practices, use of personal protective equipment, containment equipment, and laboratory design characteristics.

(4) Additional general training for handling toxins will include relevant items from (3) above plus—

(a) The availability of reference material on the hazards and safe handling of toxic substances.

(b) The biological effects of the toxins in use.

3–2. Operational prerequisites

a. Evaluation of the risks. The risk assessment of laboratory activities involving the use of etiologic agents is ultimately a subjective process. Those risks associated with the agent, as well as with any adjunct elements of the activity to be conducted, (chemicals, radioisotopes, end-products, and so forth) must be considered in the assessment.The appropriate biosafety level for work with a particular agent or animal study depends on the virulence, pathogenicity, biological stability, route of transmission, and communicability of the agent;the nature of the laboratory; the procedures and manipulations to be used; the quantity and concentration of the agent; and the availability of effective vaccines or therapeutic measures.

b. Characteristics of etiologic agents. The characteristics of etiologic agents, primary laboratory hazards of working with the agent, and recommended biosafety levels are described by CDC–NIH (HHS publication No. (NIH)88–8395), the considerations for recombinant DNA molecules are described by NIH, and those for oncogenic viruses are described by NCI–NIH (sources listed below). The commander or institute director will assign work with given etiologic agents to the appropriate biosafety level. A risk assessment should take into account not only the HHS publication No. (NIH) 88–8395, Biosafety in Microbiological and Biomedical Laboratories, as amended, but also potential hazards associated with the organism and the product of the experimentation.

(1) When established guidelines exist, these will be followed.The primary source guidelines are—

(a) HHS Publication No. (NIH) 88–8395, Biosafety in Microbiological and Biomedical Laboratories, as amended, and updates published in Morbidity and Mortality Weekly Report.

(b) NIH Guidelines for Research Involving Recombinant DNA Molecules (FR 51: 16958–16985 and updates).

(c) The publication by the American Committee on Arthropod-Borne Viruses Subcommittee on Arbovirus Laboratory Safety(SALS) entitled Laboratory Safety for Arboviruses and Certain Other Viruses of Vertebrates in the American Journal of Tropical Medicine and Hygiene, 29(6), 1980, pp. 1359–1381.

(d) The Department of Health and Human Services Publication No.(NIH) 76–1165 by the National Cancer Institute (NCI) entitled Biological Safety Manual for Research Involving Oncogenic Viruses.

(2) When samples with unidentified viable agents are obtained, a knowledgeable and qualified scientist will evaluate the risks and make recommendations to the safety officer, who will add recommendations for review and approval by the commander or institute director. When guidelines for a specific organism are not established, in addition to these steps, the CDC or SALS or both will be consulted. Their recommendations will be documented and provided to the commander or institute director before approval.

c. Selection of facilities. The facility requirements identified by the risk assessment will be adhered to. Any variations and compensatory measures will be approved by the Institutional Biosafety Committee (IBC) (when recombinant DNA molecules are involved), the safety officer, and the commander or institute director before a request for an exception or waiver is submitted as stated in AR 385–69.

d. Policies and procedures. Policies in the form of a laboratory safety manual, regulations, memorandums, or SOPs are required for work with etiologic agents in the BDP. Before beginning a new procedure, the policies and procedures will be reviewed to ascertain that the intended operations are described and to determine the requirements that apply to the operation. If procedures exist for the intended operation, personnel will be trained to follow them; if procedures do not exist, then a detailed SOP will be written, reviewed, and approved before beginning the operation. SOPs will conform to the requirements stated in paragraph 2–2 *d*, and be signed by all personnel who are required to follow the procedures, thus acknowledging that they have read and understood the contents. All SOPs that pertain to a specific area (room, laboratory, or suite) will be available at the worksite.

3–3. General laboratory techniques

The general requirements for use of etiologic agents, both toxins and viable etiologic agents, are as follows:

a. General techniques applicable to etiologic agents.

(1) A fully fastened long sleeved laboratory coat, gown, uniform, or coveralls will be worn in laboratories or animal rooms.

(2) Eating, drinking, smoking, and applying cosmetics are not permitted in the work areas.

(3) Personnel must wash their hands after they handle etiologic agents or animals, and before leaving the laboratory area.

(4) Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used.

(5) Gloves—

(a) Will be worn when manipulating etiologic agents and handling containers of etiologic agents. Gloves are not required when materials are packaged appropriately for shipment.

(b) Will be selected based on the hazards.

(c) Will be changed frequently (or decontaminated frequently), and will be decontaminated or discarded into a labeled biohazard container after each use and immediately upon observable direct contact with an etiologic agent.

(d) Will be removed at the work space (workbench or hood) after handling etiologic agents to ensure that doorknobs and other surfaces are not contaminated.

(6) Good housekeeping will be maintained. This includes—

(a) Work areas free of clutter.

(b) Work environment free of tripping hazards, with adequate access to exits, emergency equipment, controls, and such.

(c) Benches and general work areas will be cleaned regularly using a wet sponge or similar method with disinfectant as appropriate. Methods that stir up dust such as sweeping or using vacuum cleaners, (except for HEPA filtered vacuum cleaners) are unacceptable.

(d) Specific work areas will be cleaned and decontaminated immediately following each use of an etiologic agent (at least once a day) and after any spill of viable material.

(e) Hallways and stairways will not be used for storage.

(7) All solutions, reagents, and chemicals will be labeled.

(8) All contaminated liquid or solid wastes will be inactivated before disposal.

(9) Work will be conducted over spill trays or plastic-backed absorbent paper. The paper will be removed, decontaminated, or disinfected, and the general area wiped with decontaminant at the end of each work day or at the end of the experiment, whichever occurs first.

(10) Etiologic agents will be kept in closed containers when not in use. Cultures, solutions, or dried etiologic agents in glass vessels transported or incubated within a room or suite will be handled in nonbreakable, leak-proof pans, trays, pails, carboys, or other secondary containers large enough to contain all the material, if the glass vessel leaks or breaks. Etiologic agents removed from a room or suite for transport to another approved area within the same building will be placed in a closed unbreakable secondary container before removal from the laboratory. The secondary container will be labeled on the exterior with a biohazard symbol and identification of the contents, including the required biosafety level, the scientific

name, the concentration (if applicable), and the responsible individual. The secondary containers will be wiped with suitable disinfectant before removal from the laboratory or area.

(11) Working stocks of etiologic agents will be stored in double containers. The primary and secondary containers will provide a positive seal and the secondary container will be unbreakable. The secondary container will be labeled as stated in (10) above and with the date stored.

(12) Storage units (for example, freezers, refrigerators, cabinets, and hoods) will be labeled with the universal biohazard sign and indicate the classes of etiologic agents contained in them.Storage units will be secured when not in use.

(13) All contaminated materials, containers, spills, and solutions will be decontaminated or disinfected by approved methods before disposal.

(14) After injection of an etiologic agent into animals, the site of injection will be swabbed with a decontaminant.

(15) Syringes.

(a) Reusable or disposable syringes will be of the fixed needle or LUER-LOK type (or equivalent) to assure that the needle cannot separate during use.

(b) After use, nondisposable glass syringes with attached needles contaminated with etiologic agents will be submerged in a container of decontaminant. Disposable syringes will be discarded with needles attached in puncture-proof rigid containers. Needles will not be recapped after use.

(c) Sterilized or decontaminated containers marked 'SYRINGES AND/OR NEEDLES' may be deposited in appropriate refuse containers after proper packaging and destruction of the contents. Needles or syringes may not be destroyed by clipping. A mechanical shear may be used to smash or shear needles after or concurrently with sterilization or decontamination.

[Note: Many States, especially those on the Eastern seaboard, have implemented strict requirements for the disposal of medical wastes. For example, Maryland has designated all waste from a microbiological laboratory as hazardous waste with licensing requirements for disposal. There are rigid documentation requirements for generators of 50 kilograms per month or more of waste, while all medical waste released for transport off-site must be manifested to a State licensed medical waste hauler with the destination specified. Additionally, in some cases, the local government (for example, a city) regulates the disposal of these wastes. These requirements will be identified and followed.]

(16) Refrigerators, deep freezers, and dry ice chests should be checked, cleaned out, and defrosted periodically to remove any ampules, tubes, and so forth, containing etiologic agents that may have broken during storage. Rubber gloves and respiratory protection appropriate to the materials in storage should be worn during cleaning. Do not store flammable solutions in nonexplosion proof refrigerators.

b. Additional techniques applicable to work with viable etiologic agents. The major objective of these techniques is to assist in protection against laboratory acquired infections. Air sampling studies have shown that aerosols are generated from most of the manipulations of bacterial and viral cultures common to research laboratories. The generation of aerosols during routine laboratory manipulations must be considered when evaluating the individual degree of risk, keeping in mind the four main factors governing infection: dosage, virulence of the organism, route of infection(for example, skin, eyes, mouth, lungs), and host susceptibility(for example, state of health, natural resistance, previous infection, response to vaccines and toxoids). The requirements stated below are minimum handling requirements to prevent accidental infection created by incidental aerosols.

(1) All procedures are performed carefully to minimize the creation of aerosols.

(2) No infectious mixtures will be prepared by bubbling air through a liquid.

(3) Pipettes.

(a) No infectious material will be forcibly ejected from pipettes. Only to deliver (TD) pipettes will be used.

(b) Pipettes used with infectious or toxic materials will be plugged with cotton unless they are used exclusively in a gas-tight cabinet system.

(c) Contaminated pipettes will be placed horizontally in a rigid container containing enough disinfectant for complete immersion. Cylinders used for vertical discard are not recommended.The container and pipettes must be autoclaved as a unit and replaced by a clean container containing fresh disinfectant.

(d) Pipetting devices must be used. Under no circumstances is mouth pipetting permitted.

(4) Syringes.

(a) Using syringes and needles for making dilutions of etiologic agents is not recommended.

(b) When removing a syringe and needle from a rubber stopper bottle containing viable etiologic agents, an alcohol soaked pledget around the stopper and needle will be used.

(c) Excess fluid and bubbles should be expelled from syringes vertically into a cotton pledget soaked with disinfectant or into a small bottle containing disinfectant soaked cotton.

(d) The site of injection of an animal will be swabbed with a disinfectant before and after injection.

(e) After use, syringes contaminated with residual infectious fluid will be submerged in a container of disinfectant in a safety cabinet prior to removal for autoclaving. To minimize accidental injection of infectious material, the removable needles should remain on reusable syringes until after autoclaving. When possible, syringes with attached needles should be placed in a pan separate from that holding other discarded materials.

(5) Centrifuges and shakers.

(a) Before centrifuging, tubes, rotors, seals, and gaskets will be checked for cleanliness and integrity. In low speed clinical-type centrifuges, a germicidal solution may be added between the tube and trunnion cup to disinfect the outer surfaces of both and to cushion against shocks that might break the tube. Metal or plastic tubes(other than nitro-cellulose) will be used.

(b) Decanting from centrifuge tubes will be avoided. If decanting is necessary, the outer rim will be wiped with a disinfectant after decanting so that material on the lip cannot spin off as an aerosol. Centrifuge tubes will not be filled beyond the level the manufacturer recommends.

(c) Broth cultures will be shaken in a manner that avoids wetting the plug or cap.

(6) Water baths in which viable etiologic agents are incubated must contain a disinfectant. For cold water baths, 70 percent propylene glycol is recommended. The disinfectant should be changed frequently.

(7) When a laboratory vacuum is used to manipulate viable etiologic agents, a secondary reservoir containing disinfectant and a HEPA filter must be employed to ensure that the laboratory vacuum lines do not become contaminated.

(8) Test tubes.

(a) Tubes containing viable etiologic agents should be manipulated with extreme care. Studies have shown that simple procedures, such as removing a tube cap or transferring an inoculum, can create a potentially hazardous aerosol.

(b) Tubes and racks of tubes containing biohazardous material should be clearly marked. All work will be conducted in biological safety cabinets. The individual employee must ensure that tubes containing biohazardous material are properly sterilized prior to disposal or glassware washing. Safety test tube trays should be used in place of conventional test tube racks to minimize spillage from broken tubes. When safety test tube trays are not used, the conventional test tube racks will be placed in a tray large enough to contain any potential spill. A safety test tube tray is one having a solid bottom and sides deep enough to hold all liquids, should a test tube break.

(9) Care should be exercised when using membrane filters to obtain sterile filtrates of viable etiologic agents. Due to the fragility

of the membranes and other factors, such filtrates cannot be considered noninfectious until laboratory culture or other tests have proven their sterility.

(10) The preparation, handling, and use of dry powders of viable etiologic agents in open containers presents unusual hazards. The slightest manipulation of such powders can cause the generation of aerosols containing a high concentration of etiologic agents.Therefore, work with dry powders of etiologic agents in open containers should be carried out in gas-tight biological safety cabinets.

3–4. Biosafety level 1

a. Requirements beyond those for all etiologic agents. BL–1 operations follow the general techniques described in paragraphs 3–3 *a* and 3–3 *b*.

b. Additional laboratory requirement. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.Examples of suitable containers are metal tubs with lids or plastic bags that are sealed and then placed inside a rigid container for transport.

c. Additional animal requirements.

(1) Bedding materials from animal cages will be removed in such a manner as to minimize the creation of aerosols. These materials will be disposed of so as to comply with applicable institutional or local requirements.

(2) Cages will be washed manually or in a cagewasher.Temperature of final rinse water will be a minimum of 180° F.

(3) Laboratory coats, gowns, or uniforms worn in animal rooms will not be worn in other areas.

3–5. Biosafety level 2

a. Additional requirements. In addition to the general microbiological techniques stated in paragraph 3–4, BL–2 operations include the following requirements:

(1) When etiologic agents are in use, a hazard warning sign incorporating the universal biohazard symbol is posted on the access door of the work area. The hazard warning sign identifies the etiologic agent, lists the name and telephone number of the institute director or other responsible persons, and indicates the special requirements for entering the laboratory.

(2) Animals not involved in the work being performed are not permitted in the laboratory.

(3) Special care is taken to avoid skin contamination with the etiologic agents; gloves will be worn when handling etiologic agents or infected animals.

(4) All wastes from laboratories and animal rooms are decontaminated before disposal.

(5) Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles.

(6) Spills and accidents which result in a potential exposure to etiologic agents will be reported immediately to the safety officer, the project leader, and the institute director.

(7) Biological safety cabinets (Class I or II) will be used when—

(a) Procedures with a high potential for creating infectious aerosols are conducted.

(b) High concentrations or large volumes of etiologic agents are used.

(8) Laboratory coats, gowns, smocks, or uniforms will be removed before leaving the animal facility or laboratory area.

b. Additional animal requirements.

(1) Cages must be decontaminated, preferably by autoclaving, before they are cleaned and washed.

(2) Approved molded masks are worn by all personnel entering animal rooms housing nonhuman primates.

(3) If floor drains are provided, the drain traps will be kept filled with water or a suitable disinfectant.

3–6. Biosafety level 3

a. Additional requirements. In addition to the requirements stated in paragraphs 3–4 and 3–5 above, the following requirements apply:

(1) Approved molded masks or respirators with HEPA filters are worn by all personnel in rooms housing infected animals.

(2) Protective clothing worn in a laboratory or animal room will be removed before exiting the laboratory or animal room.

(3) Clothing worn in laboratories and animal areas to protect street clothing will be decontaminated before being laundered.

b. Additional laboratory requirements.

(1) Laboratory doors will be kept closed.

(2) All activities involving etiologic agents will be conducted in biological safety cabinets (Class I, II, or III) or other physical containment devices within the containment module. No work in open vessels is conducted outside a biological safety cabinet.

(3) The work surfaces of biological safety cabinets and other containment equipment will be decontaminated after work with etiologic agents. Plastic backed paper toweling should be used on nonperforated work surfaces within biological safety cabinets to facilitate clean-up.

c. Additional animal requirements.

(1) Cages are autoclaved before bedding is removed and before they are cleaned and washed.

(2) Gloves are removed aseptically and autoclaved with other wastes before being disposed of or reused.

(3) Boots, shoe covers, or other protective footwear and disinfectant foot baths must be available and used when indicated.

(4) Personal protective clothing and equipment and other physical containment devices are used for all procedures and manipulations of etiologic agents or infected animals. The risk of infectious aerosols from infected animals or their bedding will be reduced by housing animals in partial containment caging systems as described in paragraph 8–8.

d. Work with BL–3 etiologic agents that require additional secondary containment. Facilities in which work with certain viruses, for example, Rift Valley fever, yellow fever, and Venezuelan equine encephalitis, is conducted require HEPA filtration of all exhaust air prior to discharge from the laboratory. All persons working with those agents for which a vaccine is available should be immunized.

3–7. Biosafety level 4

Laboratory work at BL–4 must follow the requirements stated in paragraphs 3–4, 3–5, and 3–6, as well as the following:

a. All activities are conducted in Class III biological safety cabinets or in Class I or II biological safety cabinets in conjunction with a one-piece positive pressure personnel suit ventilated by a life-support system.

b. Biological materials to be removed from the Class III cabinet or from the maximum containment laboratory in a viable or intact state must be transferred to a sealed nonbreakable primary container, enclosed in a nonbreakable sealed secondary container, and removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

c. No materials, except for biological materials that are to remain in a viable or intact state, are removed from the maximum containment laboratory unless they have been autoclaved or decontaminated before they leave the facility. Equipment or material which might be damaged by high temperature or steam is decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

d. Personnel may enter and leave the facility only through the clothing change and shower rooms. Personnel must shower each time they leave the facility. Personnel may use the airlocks to enter or leave the laboratory only in an emergency.

e. Street clothing must be removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jumpsuits, shoes, and gloves, will be provided and must be used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When leaving the laboratory and before

proceeding into the shower area, personnel must remove their laboratory clothing and store it in a locker or hamper in the inner change room.

f. When etiologic agents or infected animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biohazard symbol must be posted on all access doors.The sign must identify the agent, list the name of the commander or institute director or other responsible persons, and indicate any special requirements for entering the area (for example, the need for immunizations or respirators).

g. Supplies and materials needed in the facility are brought in by way of the double doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated after each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

h. Materials (for example, animals and clothing) not related to the experiment being conducted are not permitted in the facility.

i. Whenever possible, avoid using any glass items.

3–8. Toxins

The laboratory facilities, equipment, and procedures appropriate for work with toxins of biological origin must reflect the intrinsic level of hazard posed by a particular toxin as well as the potential risks inherent in the operations performed. All toxins must be considered to pose a hazard in an aerosol form. However, most toxins exert their effects only after parenteral exposure or ingestion, and a few toxins present a dermal hazard. In general, toxins of biological origin are not intrinsically volatile. Thus, the laboratory safety precautions appropriate for handling these materials closely parallel those for handling infectious organisms.The requirements in this section for the laboratory use of toxins of biological origin include the requirements in paragraph 3–3 *a* and the following:

a. Vacuum lines. When vacuum lines are used with systems containing toxins, they will be protected with a HEPA filter to prevent entry of toxins into the lines (or sink drains when water aspirators are used).

b. Preparation of concentrated stock solutions and handling closed primary containers of dry toxins. Preparation of primary containers of toxin stock solutions and manipulations of closed primary containers of dry forms of toxins will be conducted—

(1) In a chemical fume hood, a glove box, or a biological safety cabinet or equivalent containment system approved by the safety officer.

(2) While wearing eye protection if using an open-fronted containment system.

(3) Ensuring that gloves worn when handling toxins will be disposed of as toxin waste, with decontamination if required.

(4) With the room door closed and posted with a universal biohazard sign, or other sign, indicating that toxin work is in progress. Extraneous personnel will not be permitted in the room during operations.

(5) Ensuring that toxins removed from hoods or biological safety cabinets are double contained during transport.

(6) After verification of hood or biological safety cabinet inward airflow is made by the user before initiating work.

(7) Within the operationally effective zone of the hood or biological safety cabinet.

(8) Ensuring that nondisposable laboratory clothing is decontaminated before release for laundering.

(9) Ensuring that all individuals who handle toxins wash their hands upon each exit from the laboratory.

(10) With two knowledgeable individuals present whenever more than an estimated human lethal dose is handled in a syringe with a needle. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.

c. Manipulations with open containers of dry forms of toxins. Handling dry forms of toxins in uncovered containers (for example, during weighing) will be performed following the requirements stated in paragraphs 3–3 *a*, and *a* and *b* . above, and the following:

(1) Manipulations will be conducted in a HEPA filtered chemical fume hood, glove box, or biological safety cabinet. In addition the exhaust may be charcoal filtered if the material is volatile.

(2) When using an open fronted fume hood or biological safety cabinet, protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) will be worn so that hands and arms are completely covered. Eye and approved respiratory protection is also required. The protective clothing will not be worn outside of the laboratory and will be disposed of as solid toxin waste.

(3) Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container will be decontaminated and placed in a clean secondary container.

(4) When toxins are in use, the room will be posted to indicate"-Toxins in Use - Authorized Personnel Only." Any special entry requirements will be posted on the entrances to the room.

(5) All operations will be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.

(6) Individuals handling toxins will wash their hands upon leaving the laboratory.

d. Additional considerations of specific toxin properties. The following requirements are in addition to the requirements stated in the paragraphs above. Determine whether the material fits *b* or *c* above, and complies with the appropriate section and the following when applicable:

(1) When handling dry forms of toxins that are electrostatic—

(a) Do not wear gloves (such as latex) that help to generate static electricity.

(b) Use glove bag within a hood or biological safety cabinet, a glove box, or a Class III biological safety cabinet.

(2) When handling toxins that are percutaneous hazards(irritants, necrotic to tissue, or extremely toxic from dermal exposure)—

(a) Gloves will be selected that are known to be impervious to the toxin and the diluent (when applicable) for the duration of the manipulations.

(b) Disposable laboratory clothing will be worn, left in the laboratory upon exit, and disposed of as solid toxin waste.

f. Aerosol exposures. The requirements found in *a* and *b*. above will be complied with plus the following:

(1) Chambers, nose-only exposure apparatus, and generation system must be placed inside a fume hood, glove box, or a Class III biological safety cabinet. Glove boxes and Class III biological safety cabinets will have HEPA filters on both inlet and outlet air ports.

(2) The atmosphere from within the exposure chamber will be HEPA filtered before release inside the hood, glove box, or cabinet.

(3) All items inside the hood, glove box, or Class III biological safety cabinet will be decontaminated upon removal.Materials such as experimental samples that cannot be decontaminated directly will be placed in a closed secondary container, the exterior of which will be decontaminated and labeled appropriately.Animals will have any areas exposed to toxin wiped clean after removal from the exposure apparatus.

(4) The interior of the hood, glove box, or cabinet containing the chamber and all items will be decontaminated periodically, for example, at the end of a series of related experiments. Until decontaminated, the hood, box, or cabinet will be posted to indicate that toxins are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.

3–9. Emergencies

a. Introduction. All laboratories will establish specific emergency plans for their facilities. Plans will include liaison through proper channels with local emergency groups and with community officials.These plans will include both the building and the individual laboratories. For the building, the plan must describe evacuation routes, facilities for medical treatment, and procedures for reporting

accidents and emergencies. The plans will be reinforced by drills. Emergency groups and community officials must be informed of emergency plans in advance of any call for assistance. See AR 385–69.

b. General emergency procedures. The following emergency procedures will be followed for laboratory accidents or incidents:

(1) Using appropriate personal protection, assist persons involved, remove contaminated clothing if necessary, decontaminate affected areas, and remove personnel from exposure to further injury if necessary; do not move an injured person not in danger of further harm. Render immediate first aid if necessary.

(2) Warn personnel in adjacent areas of any potential hazards to their safety.

(3) In case of fire or explosion, call the fire department or community fire brigade immediately. Follow local rules for dealing with incipient fire. Portable fire extinguishers will be made available with instructions for their use. Fire fighters responding to the fire scene will be advised to wear a self-contained positive pressure breathing apparatus to protect themselves from toxic combustion byproducts.

(4) Laboratories must be prepared for problems resulting from severe weather or loss of a utility service. In the event of the latter, most ventilation systems not supplied with emergency power will become inoperative. All potentially hazardous laboratory work must stop until service has been restored and appropriate action has been taken to prevent personnel exposure to etiologic agents.

(5) In a medical emergency, summon medical help immediately.Laboratories without a medical staff must have personnel trained in first aid available during working hours.

(6) For small-scale laboratory accidents, secure the laboratory, leave the area, and call for assistance.

(7) When handling mixed hazards (for example, a substance or mixture that may be infectious and radioactive, or infectious and chemically toxic), respond with procedures addressing the greater hazard first, and then follow through with those for the lesser hazards to ensure that all appropriate steps have been taken.

c. Evacuation procedures. Building and laboratory evacuation procedures will be established and communicated to all personnel.

(1) Emergency alarm system.

(a) There will be a system to alert personnel to an emergency that requires evacuation of the laboratory or building. Laboratory personnel must be familiar with the location and operation of alarm equipment.

(b) Isolated areas (for example, cold, warm, or sterile rooms)will be equipped with an alarm or communication system that can be used to alert others outside to the presence of a worker inside, or to warn workers inside of an emergency that requires evacuation.

(2) Evacuation routes will be established and an outside assembly area for evacuated personnel must be designated. All individuals should be accounted for.

(3) Shut-down and start-up procedures.

(a) Guidelines for shutting down operations during an emergency evacuation will be available in writing. Those guidelines will include procedures for handling any power failure emergency.

(b) Written procedures will also be provided to ensure that personnel do not return to the laboratory until the emergency is ended. Those procedures must also contain start-up operations for the laboratory.

(c) All shut-down and start-up procedures will be available to personnel and reviewed semiannually.

(4) All aspects of the building evacuation procedure will be tested semiannually with practice drills.

d. Spills.

(1) All areas where work with etiologic agents is performed will have designated personnel to respond to a spill and provide protective apparel, safety equipment, and materials necessary to contain and clean up the spill. Protective clothing requirements are described in paragraph 4–11. Also, there will be supplies on hand to deal with the spill consistent with the hazard and quantities of the spilled substance.

(2) The safety officer will be notified immediately of all spills. The first line supervisor will ensure that proper clean-up techniques are employed.

(3) Procedures for etiologic agents are as follows:

(a) A program for responding to spills of etiologic agents will be developed and implemented. This program will contain emergency response procedures for a biological spill, which will be tailored to the potential hazard of the material being used, the associated laboratory reagents involved, the volume of material, and the location of the materials within the laboratory. Generally, the spill should be confined to a small area while minimizing the substance's conversion to an aerosol. The spill will be chemically decontaminated or neutralized, followed by a cleanup with careful disposal of the residue. If the spilled material is volatile and noninfectious, it may be allowed to evaporate but must be exhausted by a chemical hood or ventilation system.

(b) When a mishap occurs that may generate an aerosol of etiologic agents requiring BL–2 (or higher) containment, the room must be evacuated immediately, the doors closed, and all clothing decontaminated, unless the spill occurs in a Class II or Class III biological safety cabinet. Sufficient time must be allowed for the droplets to settle and the aerosols to be reduced by the air changes of the ventilation system before decontaminating the area.The area will then be decontaminated to prevent exposure to the infectious agents or toxic substances. Reentry procedures to perform the decontamination will conform to e below.

(c) A spill of biohazardous material within a biological safety cabinet requires a special response and cleanup procedure. Cleanup will be initiated while the cabinet continues to operate, using an effective chemical decontaminating agent. Aerosol generation during decontamination and the escape of contaminants from the cabinet must be prevented. Caution must be exercised in choosing the decontaminant, keeping in mind that fumes from flammable organic solvents, such as alcohol, can reach dangerous concentrations within a biological safety cabinet.

(4) Procedures to cleanup combined radioactive and biological spills are as follows:

(a) Both the radiation protection officer (RPO) and the safety officer must be notified immediately whenever there is a spill of radioactive biological material, regardless of its size. Laboratory personnel may be expected to clean up the spill. The RPO will direct the cleanup, in accordance with the NRC license for the facility.

(b) The spill will be cleaned up in a way that minimizes the generation of aerosols and spread of contamination. All items used in cleaning up the spill must be disposed of as radioactive waste.

(c) Following cleanup, the area, affected protective clothing, and all affected equipment and supplies must be surveyed for residual radioactive contamination. All potentially affected areas and items that are not disposable will be wipe tested to verify that unfixed radioactive contamination has been removed. If fixed contamination is found, the RPO will determine the requirements for additional cleanup.

e. Reentry procedures. This section applies when reentry is necessary to clean up a spill outside of a hood or biological safety cabinet, or to decontaminate or service engineering controls that have failed or malfunctioned so that they do not provide the required containment.

(1) When agents requiring BL–1 or BL–1 LS containment are involved, the clothing requirements stated in paragraph 4–10 *a* or *b*, as appropriate, will be followed. Individuals will remove the required protective clothing when finished and wash their hands before proceeding to other tasks.

(2) When agents requiring BL–2, BL–2 LS, or toxin procedures and containment are involved, personnel will be required to wear the clothing described in paragraph 4–10 *c* or *d*, as appropriate. Outer protective clothing will be removed and left in the room before exiting and personnel will wash their hands before proceeding on to other activities.

(3) When agents requiring BL–3, or BL–3 LS containment are involved, containers for sealing up inner protective clothing and decontaminant will be placed at the room exit.Personnel will be

required to wear the clothing described in paragraph 4–10 *e* When exiting the area after decontamination procedures, individuals will remove their outer layer of protective clothing just before exiting the room. Once outside the room, the inner layer of protective clothing (for example, coverall) will be removed and placed in the container and the inner gloves will be decontaminated before being removed and placed in the container. Personnel will proceed directly to the shower facility to take a complete shower before exiting the facility.

(4) When agents requiring BL–4 containment are involved, the following applies as appropriate to the type of BL–4 facility:

(a) When a spill requiring cleanup is in an area designed for use with personal positive pressure suits, the entry and exit procedures will be those normally required to enter or exit the area.

(b) When entering a nonsuit area where a spill of etiologic agent has occurred outside the containment of a Class III biological safety cabinet, personnel will wear the clothing as described in paragraph 4–10 *f*. Before entry, decontamination areas will be established. To accomplish this, two step-in decontamination pans with the appropriate disinfectant will be set up [one just inside the room (where the contamination exists) and the second immediately outside the room].Immediately outside the room, there will also be a sealable container suitable for sealing up the suit and any air lines (if used).

(c) When exiting the room, suited individuals will place all equipment and other items in autoclaves or disinfectant, step into the disinfectant pan, and wash down the exterior of their suits with appropriate disinfectant. When completed, the door to the room will be opened and the individual will step through the doorway into the second disinfectant pan. The suit will be thoroughly rinsed with disinfectant again before moving toward the exit from the facility.The suit (but not the respirator) will be placed in the provided container. The individual will proceed through another doorway before removing the respirator and placing it in a closed container for decontamination. The individual will then proceed directly to the shower area and take a full shower before exiting the area. In case they are needed, personnel will be standing by ready to render assistance. Suited individuals will be visually observed, if possible. When visual observation is not possible, a communications system is required.

f. Mishap reports and investigations.

(1) Each institution must have a defined system for reporting laboratory injuries, illnesses, and mishaps, as well as for investigating them. These events will be documented and reported to the appropriate safety, supervisory, and occupational health personnel. Those organizations subject to the regulations promulgated by the OSHA will follow the specific requirements for reporting injuries in the work place contained in those regulations.The requirements stated in AR 385–69, State, and local government requirements for similar reporting will be followed.

(2) Forms for recording mishaps will be available and completed for all laboratory mishaps. Those reports must include a description of the mishap and any factors contributing to the it. In addition, a description of any first aid or other health care given to the employee will be included. Responsibility for completing these forms must be clearly defined in the facility safety manual. Mishaps will be reviewed periodically by the safety officer, the safety committee, the employee health unit, or other appropriate personnel.Individual reports or a summary must be sent, along with recommended changes in laboratory procedure or policy, to the commander or institute director. Policy or procedural changes must be implemented if deemed necessary by the commander or institute director.

(3) Any mishaps with etiologic agents used under sponsorship of the BDP that result in sero-conversion or a laboratory acquired illness will be reported.

3–10. Large-scale operations

a. Large-scale. In addition to the requirements stated in paragraph 3–4, the following applies to research or production activities involving viable etiologic agents in quantities greater than 10 liters:

(1) All large-scale operations will be conducted in facilities described in paragraph 7–6.

(2) Cultures will be handled in a closed system.

(3) Sample collection, the addition of materials, and the transfer of culture fluids will be done in a manner which minimizes the release of aerosols or contamination of exposed surfaces.

(4) A closed system or other primary containment equipment that has contained viable organisms will not be opened for maintenance or other purposes unless it has been sterilized.

(5) SOPs will include a section describing and requiring a validation of the process equipment's proper function.

(6) Scientists, technicians, equipment workers, and support personnel with access to the large-scale production area during its operation will be included in the medical surveillance program.

b. BL–2 LS. In addition to the requirements stated in paragraphs 3–10a and 3–5, the following procedures will be employed for BL–2 LS:

(1) Rotating seals and other mechanical devices directly associated with the closed system used for the propagation and growth of viable organisms will be designed to prevent leakage or will be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to HEPA filters or through other equivalent treatment devices.

(2) A closed system used for the propagation and growth of viable organisms and other primary containment equipment used to contain operations involving viable organisms will include monitoring or sensing devices that monitor the integrity of containment during operations.

(3) Systems used to propagate and grow viable organisms will be permanently identified. This identification will be used in all records reflecting testing, operation, and maintenance and in all documentation relating to the use of this equipment.

c. BL–3 LS. In addition to the requirements stated in paragraphs 3–10 *b* and 3–6, the following procedures apply:

(1) Personnel entry into the controlled area will be through the entry area specified in paragraph 7–6 *c*(1).

(2) Persons entering the controlled area will exchange or cover their personal clothing with work garments such as jumpsuits, long sleeved laboratory coats, pants and shirts, head cover, and shoes or shoe covers. On exit from the controlled area, the work clothing may be stored in a locker separate from that used for personal clothing, or discarded for laundering. Clothing will be decontaminated before laundering.

(3) Entry into the controlled area during periods when work is in progress will be restricted to those persons required to meet program support needs.

(4) Prior to entry, all persons will be informed of the operating practices, emergency procedures, and the nature of the work conducted.

(5) The universal biohazard sign will be posted on entry doors to the controlled area and all internal doors. The sign posted on the entry doors to the controlled area will include a statement of agents in use and personnel authorized to enter.

(6) Equipment and materials required for the management of accidents involving viable organisms will be available in the controlled area.

d. BL–4 LS. Guidelines for these operations are not established. If these are needed, they must be established by the U. S. Army Surgeon General or the NIH on an individual basis.

3–11. Operations with radioactive material

Operations that combine etiologic agents with radioactive material present unique problems. When this is the case, the following apply:

a. Radiation program. A radiation program meeting the requirements of AR 385–11 and NRC licensing that allows the particular isotope and its use is required. The requirements for acquisition, handling procedures, labeling, storage, training, monitoring, and disposal will be described in an organizational policy document.

b. Procedure approval. In addition to the required approvals for work with etiologic agents, the RPO will approve all SOPs involving the use of radioactive materials. Laboratory operators must be fully trained, with annual training updates as required by the existing license.

c. Special situations.

(1) The laboratory waste must be segregated as radioactive waste and disposed of as such after it has been decontaminated. Do not mix nonradioactive waste with radioactive waste as the disposal of radioactive waste is much more complex and expensive. When RCRA listed chemicals are mixed with radioactive waste, it becomes"mixed waste" for which there is currently no means of disposal.

(2) Activities conducted with radioisotopes should be confined to the smallest number of areas or rooms consistent with requirements.

(3) Decontamination methods specific to etiologic agents will not always remove radioactivity. Other methods, such as specialized detergents and solvents designed for this use, should be employed to remove residual radioactivity.

Chapter 4
Personal Protective Equipment

4–1. Introduction

Personal protective equipment (PPE) includes clothing and equipment used to protect the laboratory worker from contact with infectious, toxic, and corrosive agents, as well as excessive heat, fire, and other physical hazards. The appropriate PPE for any activity depends upon the proposed operations and the potential hazards associated with them. While PPE is an important item of personal protection, it serves as only a secondary line of protection against hazards in the workplace. Engineering controls(chap 8), combined with common sense, good laboratory techniques, and adherence to SOPs, are the primary barriers to exposure. There are some situations, however, in which it is either impractical or impossible to rely exclusively on engineering controls. In these cases, PPE may form the primary barrier between personnel and the hazardous or infectious materials.

4–2. Minimum laboratory attire when using etiologic agents

Individuals required to wear PPE will be trained in its proper use. The PPE listed below is the minimum required when etiologic agents are handled at any biosafety level. Research with etiologic agents usually involves hazards other than those presented by the agents themselves. When PPE is selected, the hazards presented by these other factors must be considered regardless of the biosafety level used. For example, toxic chemicals are commonly used in research involving etiologic agents. The processes may expose personnel to physical hazards, such as heat or animal bites, and the decontamination process may involve the handling of toxic or corrosive materials. When the PPE required to mitigate these hazards exceeds that of the minimum requirements, the necessary PPE will be selected considering all of the hazards. Information regarding the additional appropriate PPE worn to protect against these hazards will be available from one of the following sources: MSDS, SOP for the operation, or the safety officer. Deviations from the standards stated in approved SOPs must be approved by the safety officer. All laboratory coats worn to protect the individual should be left in the laboratory when that individual leaves. In each case, the minimum attire will be—

a. Laboratory workers. Street attire is permissible in the laboratory, but must include closed-toe shoes. A full-length, long sleeved, fully fastened laboratory coat, gown, or smock will be worn over the street attire in the laboratory at all times. The laboratory clothing will be removed and left in the laboratory when leaving to enter nonlaboratory use areas.

b. Animal caretakers. In addition to the clothing requirements in paragraph 4–2 *a* above, animal handlers will be provided with safety shoes or safety boots. The requirements of paragraph 4–4 *b* should also apply.

c. Nonhuman primate rooms. Personnel entering rooms housing nonhuman primates will wear the clothing stated in paragraph 4–2 *a*and, if applicable, paragraph 4–2 *b* in addition to a molded mask or HEPA filtered respirator, latex or vinyl gloves, and eye protection.

4–3. Biosafety level 1

This level requires only the minimum attire described in paragraph 4–2 above.

4–4. Biosafety level 2

This level requires the following additions to the minimum clothing specified in paragraph 4–2 above:

a. Laboratory. Gloves (type dependent on the application) will be worn when handling etiologic agents or containers of etiologic agents and when handling infected animals.

b. Animal rooms.

(1) Protective clothing will be changed completely every day.One- or two-piece laboratory suits or solid-front gowns and wraparound smocks are preferable. Full-length, long sleeved, fully fastened laboratory coats are allowed.

(2) Eye protection must be worn when handling nonhuman primates.

(3) Appropriate gloves must be worn.

(4) Molded masks or HEPA filtered respirators will be worn in rooms housing nonhuman primates.

4–5. Biosafety level 3

The outer clothing worn in these facilities must never be worn outside the facility. Color coded clothing that is worn only in the facility is recommended to remind individuals not to wear it outside. The minimum clothing includes—

a. Laboratory.

(1) Long sleeved, solid front, or wraparound gowns, scrub suits, or coveralls over street attire which includes closed-toe shoes. Dedicated shoes, boots, or shoe covers will be worn in the facility.

(2) Appropriate gloves.

b. Animal rooms.

(1) A complete change of protective clothing on a daily basis-.Long sleeved one- or two-piece solid front uniforms, solid-front gown, wraparound smocks, or solid front coveralls.

(2) Eye protection must be worn when handling nonhuman primates.

(3) Molded masks or HEPA filtered respirators will be worn in rooms housing infected animals.

(4) Shoe covers will be worn and removed before exiting the room; alternatively, disinfectant footbaths will be used for each exit from the room when infected animals are present.

4–6. Biosafety level 4

Street clothing must be removed in an outer clothing change room and kept there. Clothing worn in the facility will be removed in an inner change room and a shower taken before dressing in street clothing. Two distinct PPE requirements exist for BL–4 operations:

a. Class III biological safety cabinet containment. Clothing requirements when all etiologic agents and infected animals are housed and manipulated in Class III biological safety cabinets will include—

(1) Complete change of clothing and wet shower upon exit. This includes undergarments, pants and shirts or jump suits, and shoes.While it is preferred that the shower include washing the hair, head covers will be worn by those who do not wash their hair on each exit.

(2) Appropriate inner gloves. The inner gloves will be donned in the change room.

b. Class I or II biological safety cabinet containment. Clothing requirements for this level when etiologic agents are contained in Class I or II biological safety cabinets or equivalent partial-containment caging systems (for infected animals)(see paras 8–8 and 8–9) include—

(1) Complete change of clothing and wet shower upon exit. This includes undergarments, pants and shirts or jump suits, and shoes.While the shower should include washing the hair, head

covers will be worn by those who do not wash their hair on each exit.

(2) Appropriate inner gloves donned in the change room.

(3) A one-piece positive pressure suit described in paragraph 4–11 *g*.

(4) Impervious boots fitted over the suit.

4–7. Large-scale operations

The clothing requirements for these are the same as for the corresponding biosafety levels for laboratory operations.

4–8. Solutions of toxins and dry forms of toxins in closed containers

In addition to the minimum clothing specified in paragraph 4–2 above, disposable gloves or gloves designed to protect against the diluent will be worn when handling these materials.

4–9. Dry forms of toxins handled in open containers

In addition to the requirements stated in paragraph 4–8 above, the requirements stated in paragraph 3–8 *c* apply.

4–10. Situations specified in paragraph 3–9e

The clothing requirements for this section are for the emergency procedures specified in paragraph 3–9e. Because mishaps can occur and there are no feasible or available means to mitigate the potential hazard adequately by engineering controls, the clothing requirements exceed those required for a properly conducted laboratory operation at an equivalent biosafety level. The protective equipment required will be selected based upon an assessment of the potential hazards that could be encountered. The following clothing requirements are given as a guide. The selection of PPE will be based upon the highest possible level of contamination that could exist in the room. This will be based upon what is known about the operations that were conducted in the room during and prior to the current incident. In each situation, the aerosols will be allowed to dissipate or settle before entry(approximately 30 minutes). The following clothing requirements apply to these situations:

a. BL–1.

(1) Gloves.

(2) Outer complete covering such as a pair of coveralls.

(3) Shoe covers, provided shoes, or safety shoes or boots.

(4) Eye protection (maintenance only).

b. BL–1 LS. The same as described in paragraph 4–10 *a* with the following additions:

(1) An impervious apron.

(2) Impervious boots.

c. B–2 and toxins.

(1) Gloves.

(2) Full outer covering such as a coverall.

(3) Shoe covers, provided shoes, or safety shoes or boots(maintenance).

(4) An approved half-face or full-face respirator with HEPA filters (worn).

(5) Eye protection.

(6) An impervious apron (not required for entry only).

d. BL–2 LS. The same as paragraph 4–10 *c* with the addition of impervious boots.

e. BL–3 and BL–3 LS.

(1) A complete change of clothing.

(2) Gloves.

(3) An approved full-face HEPA or HEPA plus charcoal filtered respirator.

(4) An impervious apron (not required for entry only).

(5) Impervious boots.

(6) Head cover.

f. BL–4.

(1) A full change of inner clothing.

(2) An inner pair of gloves.

(3) A one-piece positive pressure suit as described in paragraph 4–11 *g*, or a one-piece suit with an approved positive pressure self-contained breathing apparatus (SCBA)and an supplied air respirator (SAR) or both (see para 4–11 *f*).

(4) Appropriate gloves fitted to the suit.

(5) Impervious boots fitted over the suit.

4–11. Specific requirements for individual personal protective equipment items

a. Aprons. Simple plastic or rubber aprons.

b. Boots. When boots must be worn with an apron, the apron should cover the boot tops sufficiently so that liquids splashed on the apron will not run into the boots.

c. Eye and face protection. Eye protection will meet or exceed the requirements of OSHA found in the 29 Code of Federal Regulations (CFR) 1910.133 and will be worn at all times when required. Special eye wear may be required around ultraviolet (UV) light source.

d. Gloves.

(1) No one glove will be satisfactory for all applications.Gloves are fabricated in a wide assortment of materials. The type of glove selected will depend upon the specific activity. The various activities in biocontainment facilities call for gloves to protect against etiologic agents in situations where micro-manipulations are required and excellent tactile feed-back through gloves is important, gloves for handling hot glassware and cryogenic materials, and gloves to protect against animal bites, toxic substances, chemical carcinogens, solvents, acids, and caustics.Many of these requirements call for gloves distinctly different from gloves suitable for the other hazards. As a result, the SOP for each operation should address these hazards and specify the appropriate glove required for each operation. Consult MSDSs, manufacturers'glove charts, and the safety officer to determine the correct glove type needed.

(2) Before donning a pair of gloves, examine them closely to ascertain that they are in serviceable condition. Check for rips and pin holes. Gloves should over-wrap the cuff and lower sleeve of the laboratory garment.

(3) Operations in open-front biological safety cabinets should be planned so that once the operator has inserted gloved hands into the cabinet, he or she does not have to withdraw them from the cabinet until the work has been completed. If gloves become visibly contaminated, they will be removed and decontaminated. Additional gloves should be available so that work can continue. When wearing gloves for an extended period, change them periodically or decontaminate them. Individual SOPs will designate the appropriate period based upon the hazards.

(4) Gloves will be removed before going from one level of containment to another (remove gloves in a safety cabinet before removing your hands from the cabinet). Take care to ensure that skin is not touched with the outer surface of contaminated or potentially contaminated gloves when they are removed. Gloves will be placed in suitable decontaminant when they are removed. Disposable gloves will be placed in a covered container for decontamination or disposal.

(5) Gloves that are a part of a Class III biological safety cabinet system will be examined initially and after each sterilization of the Class III biological safety cabinet system. The Class III biological safety cabinet will be pressure tested at least annually by the soap bubble/halogen test as prescribed in NSF Standard No. 49, Appendix B1 (latest revision June 1987), and certified when the HEPA filter units are serviced.

(6) Sterilization of nondisposable gloves either before use or before reuse is usually done with ethylene oxide or formaldehyde gas. Sterilized gloves must be aerated in flowing sterile (filtered)air at 21 degrees Celsius or higher for a minimum of 24 hours prior to use to prevent skin burns and irritation from residual decontaminants.

e. Laboratory clothing. Users will check clothing before wearing it to ensure that it is free from defects that would compromise its usefulness.Laboratory clothing (except BL-1) will be decontaminated before being released for laundering by untrained or unprotected personnel.Protective laboratory clothing that requires the wearer

to pull it over the head will not be used. Laboratory clothing will meet OSHA requirements found in 29 CFR 1910.132.

f. One-piece suits. One-piece suits with a respirator under the suit are not used to any great extent except in certain emergencies. The respirators used with these are supplied air by an approved positive pressure SCBA or SAR. Respirators will be of the pressure-demand or constant flow type. The air provided will meet OSHA requirements found in 29 CFR 1910.134, the requirements of Grade D breathing air as specified in the Compressed Gas Association pamphlet G–7.1 and American National Standards Institute (ANSI) Z86.1–1973.When a suit is used in an area that does not have a chemical shower to decontaminate it, a decontamination station will be set up for this purpose. Suits maintained for emergency use will be inspected at least quarterly and respiratory equipment will be inspected monthly.

g. One-piece positive pressure suits. A life-support system will be provided with alarms and emergency backup breathing tanks. The air provided will be HEPA filtered meeting OSHA requirements found in 29 CFR 1910.134, the requirements of Grade D breathing air as specified in the Compressed Gas Association pamphlet G–7.1 and ANSI Z86.1–1973. A HEPA filter will be in-line between the disconnect on the suit and the breathing space in the suit. When these are used in other than an emergency situation, a chemical shower must be provided to decontaminate the surfaces of the suit as the worker leaves the containment area. Suits will be inspected before each use to check for indications of significant wear or leakage. The suits will be worn with impervious boots over the foot area of the suit and the outer gloves will be attached over the hand portion.

h. Respiratory protection equipment.

(1) Respirators and their use will be approved by the safety officer. The selection will be based on the conditions of the activities and the risks involved. In general, National Institute for Occupational Safety and Health (NIOSH) approved respirators that use aerosol filters for dusts and fumes having a Threshold Limit Value (TLV) of less than 0.05 mg/m3 have been found acceptable for use in microbiological laboratories. Alternatively, the Army M–17 or M–9 masks may be used. Air-supplied hoods are used in situations where greater respiratory protection is required without the need for body protection. One-piece suits are used when total body and respiratory protection are required.

(2) When respirators are used, a respirator protection program will be established that conforms to AR 11–34 and OSHA standards in 29 CFR 1910.134. In general, a medical authority will designate who is to wear respirators, they will be fitted by individuals trained in their use and limitations, and wearers will be responsible for the proper storage and regular inspection of their assigned respirators. Air purifying respirators will not be worn in oxygen deficient environments.

(3) Reusable respirators that have been worn in a contaminated area will be decontaminated before reuse. At the end of each workday when a respirator has been worn in an area where it was required, the wearer will wipe it down with an appropriate liquid decontaminant. A damp cloth soaked in the decontaminant, with the excess liquid squeezed out, will be used for the wipe-down process, taking care to ensure that all crevices are reached. The respirator will be rinsed with clean, warm water. Visibly contaminated respirators will be decontaminated and discarded.

(4) Respirator programs will comply with AR 385–10 and AR 11–34.

i. Shoes. All shoes specially issued for use in controlled access areas should be identified so that they can be segregated from other areas. Safety shoes or boots meeting OSHA requirements stated in 29 CFR 1910.134 will be issued wherever heavy items or corrosive chemicals are handled. These will be sterilized appropriately after visible contamination. In certain situations (excluding BL–4 operations), it is desirable to wear disposable booties over street shoes, especially when product protection is required.

Chapter 5
Decontamination and Disposal

5–1. Introduction

All material or equipment that is potentially contaminated with etiologic agents must be rendered nonhazardous before disposal.This chapter describes the acceptable physical and chemical decontamination methods and the general applicability of each. In general, all infectious materials and all contaminated equipment or apparatus will be sterilized before being washed and stored or discarded.

5–2. Methods of decontamination

a. Autoclave. The use of wet heat is the most dependable procedure for destroying all forms of microbial life. An autoclave employs saturated steam under a pressure of approximately 15 pounds per square inch (psi) to achieve a chamber temperature of at least 121 degrees Celsius for a minimum of 15 minutes. The time is measured after the temperature of the material being sterilized reaches 121 degrees Celsius. Other combinations of temperature and pressure(some of which are dependent on the equipment used) can be used to accomplish sterilization provided that the efficacy of sterilization is validated as described below. The most critical factor in ensuring the reliability of this sterilization method, other than proper temperature, is preventing entrapped air that is not replaced by steam. Material to be autoclaved must come in contact with steam and heat and, as a result, it may be necessary to add water to a load of waste to aid in the formation and penetration of steam.Autoclaves use either a steam activated exhaust valve that remains open during the replacement of air by live steam until the steam triggers the valve to close, or a precycle vacuum to remove air prior to steam introduction.

b. Sterilization. Sterilization will be verified using biological indicators(for example, Bacillus *stearothermophilus* spores)at locations throughout the autoclave, to include placement in the center of test loads, when the autoclave is first put into service, and after any maintenance or repairs. The primary means of verifying routine sterilization will be through using chemical indicators (for example, autoclave tape or labels) at locations throughout the autoclave. In addition each autoclave will be equipped with a permanent means to record time and the temperature of each operational event as a means of ensuring sterilization. The type of materials being handled must be reviewed and standard conditions for sterilization of each established. As a guide, the manufacturer's manual for the autoclaves will be consulted as a starting point in establishing these conditions. Treatment conditions to achieve sterility will vary in relation to the volume of material treated, the contamination level, the moisture content, and other factors that should be considered and which may cause the times to lengthen.In each case, the conditions will be established based on tests which verify that the conditions selected are effective. In addition to being effective for viable agents, autoclaving effectively inactivates most protein toxins.

c. Dry heat. Dry heat requires longer times or higher temperatures or both than does wet heat. If used, the specific sterilization times and temperatures must be determined for each type of material being sterilized. In general, sterilization by dry heat can be accomplished at 169–170 degrees Celsius for periods of 2 to 4 hours. Higher temperatures reduce the time requirements. The heat transfer properties and spatial relation or arrangement of materials in the load are critical in ensuring effective sterilization.

d. Liquid disinfectants. Liquid disinfectants may be used in surface treatment, in dip tanks, and, at sufficient concentration, as sterilants of liquid waste for final disposal. If liquid disinfectants are used, they must have been shown to be effective against the organisms present.Important considerations include: temperature, time of contact, the negative logarithm of hydrogen ion concentration (pH),

concentration and state of dispersion, penetrability, and reactivity of organic material at the site of application. Small variations in these factors may make large differences in the effectiveness of disinfection, so complete reliance should not be placed on liquid disinfectants when the end result must be sterility. If evidence of efficacy under the proposed procedures has not been reported previously, preliminary studies to verify the efficacy of liquid disinfectants must be conducted. Such studies may include attempts to recover and quantitate the agent in question from liquid or swab samples, or sealed patches, by animal inoculation, plaque assay, agar or broth cultivation, and similar methods, following controlled decontamination under the same experimental conditions envisioned for the proposed studies.

(1) Alcohol. Ethyl or isopropyl alcohol at a concentration of 70–85 percent by weight will denature proteins but is slow in its germicidal action. Alcohols are effective disinfectants for lipid containing viruses. These alcohols exhibit no activity against bacterial spores.

(2) Phenolic compounds. These are effective disinfectants against vegetative bacteria, including *Mycobacterium tuberculosis*, fungi, and lipid containing viruses. The phenolics are not effective against bacterial spores or non-lipid containing viruses. The concentrations used will be in accordance with the manufacturer's recommendations.

(3) Formaldehyde solutions. Formaldehyde in solution at a concentration of 8 percent (formalin) is effective against vegetative bacteria, spores, and viruses. It loses considerable disinfectant activity below room temperature. Due to the toxic properties of formaldehyde, the use of formalin is restricted to surfaces or materials that are contained within appropriate engineering controls.

(4) Quaternary ammonium compounds. These cationic detergents are strongly surface-active. They lose effectiveness in the presence of proteins and are neutralized by anionic detergents, such as soap.At low concentrations, they are bacteriostatic, tuberculostatic, sporostatic, fungistatic, and algistatic. At medium concentration, they are bactericidal, fungicidal, algicidal, and virucidal against lipophilic viruses. They are not tuberculocidal, sporicidal, or virucidal against hydrophilic viruses, even at high concentrations.The manufacturer's recommended dilution will be used.

(5) Chlorine. Sodium hypochlorite is normally used as a base for chlorine disinfectants. Free available chlorine is the active ingredient and, at concentrations of at least 2,500 parts per million (ppm) (0.25 percent), is a disinfectant that is active against most microorganisms and bacterial spores. Chlorine solutions at 2.5 percent free available chlorine are effective against most toxins. Chlorine solutions lose strength if exposed to air, so fresh solutions must be prepared whenever the free chlorine content falls below desired minimums.

(6) Iodine. The characteristics of chlorine and iodine are similar. Iodophor compounds with 1,600 ppm free available iodine provide a relatively rapid inactivation of all microorganisms, including some bacterial spores. A commonly available iodophor is Wescodyne. The manufacturer of Wescodyne recommends a range of dilution from 1 to 3 ounces per 5 gallons of water, giving a solution containing from 25 to 75 ppm of free iodine. At these concentrations, available iodine may be rapidly taken up by any extraneous protein present and will not be an effective sporocide. A solution providing 1,600 ppm iodine is recommended for hand washing or for use as a sporocide.

(7) Mercurials. Although the mercurials exhibit good activity against viruses, they are toxic and are not recommended for general use. They have poor activity against vegetative bacteria and are totally ineffective sporicides. The dilution recommendations stated by the manufacturer will be followed.

e. Vapors and gases. Formaldehyde, ethylene oxide, peracetic acid, beta-propiolactone, methyl bromide, and glutaraldehyde have all been used successfully as space sterilants where they can be employed in closed systems and with controlled conditions of temperature and humidity. Of these, methyl bromide, beta-propiolactone, and glutaraldehyde are not recommended because of their toxic properties. Peracetic acid can readily decompose with explosive violence in a concentrated state and must be used only in a diluted state and with extreme care. Formaldehyde and ethylene oxide are both regulated by OSHA for their potential human carcinogenicity, but do have permissible exposure levels (unlike beta-propiolactone, for example) and can be used safely under controlled conditions.

(1) Formaldehyde. Formaldehyde gas is, in general, the chemical of choice for space disinfection. Biological safety cabinets and associated effluent air handling systems and air filters, incubators, laboratory rooms, buildings, or other enclosed spaces can be disinfected with formaldehyde. The procedures found in appendix E of the National Sanitation Foundation Standard Number 49 will be followed for the disinfection of biological safety cabinets.Other enclosures or areas will be disinfected by following the same principles.

(a) To disinfect rooms, the generation of formaldehyde gas from heating powdered or flake paraformaldehyde is the preferred method.When area decontamination is performed, use 0.3 grams of paraformaldehyde for each cubic foot of space to be treated.

(b) The room or area must be above 70 degrees Fahrenheit, the relative humidity above 70 percent, and the exposure time at least 4 hours (overnight is preferred).

(c) After the required time for disinfection, the room must be cleared of the formaldehyde gas (a small room with nonporous surfaces and no materials or equipment in the room can be cleared of all detectable formaldehyde by aeration for one hour, while larger areas with equipment in them may take a full day).

(d) Before formaldehyde is used as a space disinfectant, the area to be treated must be surveyed to ensure that there are no open containers of any acidic solution containing chloride ion in order to prevent the possible formation of bis(chloromethyl)ether, a human carcinogen.

(e) Specific OSHA requirements for posting of rooms and equipment, personnel protection, and other requirements are found in 29 CFR 1910.1048.

(2) Ethylene oxide (EtO). EtO sterilization will only be conducted in a sterilizer designed for that purpose and designed to maintain potential exposure levels below the current OSHA standard.EtO is effective against all microorganisms, including spores, molds, pathogenic fungi, and highly resistant thermophilic bacteria.All materials to be used in contact with human skin (for example, clothing, shoes, masks, adhesive tape) must be aerated for at least 24 hours after sterilization and prior to use. Concentrations of 500 to 1000 ppm are required for sterilization. Specific OSHA requirements for the use of ethylene oxide are found in 29 CFR 1910.1047.

f. UV radiation. UV radiation at a wave length of 253.7 nanometers is a practical method for inactivating airborne viruses, mycoplasma, bacteria, and fungi. The usefulness of UV radiation on exposed surfaces is limited by its low penetrating power. UV radiation will only be relied upon to sterilize surfaces when conventional methods, such as autoclaving or the use of liquid disinfectants, would make the product unusable. An example is data sheets that must be brought out of a biocontainment facility. The UV intensity must be at least 40 microwatts/cm3 on the surface to be treated. Single sheets of paper may be treated by exposing them to this radiation for a minimum of 15 minutes. A calibrated photoelectric UV intensity meter, capable of measuring UV radiation at a wave length of 253.7 nanometers, will be used whenever a new UV source is installed, and quarterly thereafter, to ensure the UV source is providing at least 40 microwatts/cm3 at the work surface. Bulbs should be cleaned routinely to remove any accumulated dust and prolong bulb performance and assure proper energy output. Protective eye wear and clothing may be necessary when working around UV radiation.

5–3. Disposal

Inactivation is the first step in the disposal of etiologic agents or materials that are potentially contaminated with them. All contaminated or potentially contaminated materials must be effectively disinfected or sterilized by an approved procedure discussed in

paragraph 5–2. After decontamination, reusable items, such as clothing or glassware, may be washed with other uncontaminated or decontaminated items.

a. Combustible items. Combustible disposable items should be bagged and incinerated in an appropriate approved incinerator or otherwise disposed of in accordance with State and local regulations.

b. Noncombustible disposable items. Items will be packaged as stated in 3e below and disposed of by a licensed waste hauler.

c. Equipment. Equipment that cannot be autoclaved will be decontaminated by gaseous sterilization or with a suitable liquid disinfectant.Such equipment will be certified as decontaminated by the safety officer.

d. Waste. Materials generated, such as solvents, acids, chemical carcinogens, radioactive isotopes, medical waste, or dead animals must be decontaminated, packaged, and then disposed of in accordance with EPA, NRC, local, State, and Federal regulations.

e. Mixed waste. When two or more hazardous materials are mixed together, the mixture will be decontaminated and disposed of in accordance with EPA, NRC, State, and Federal regulations for the mixture, or for the most hazardous material.

f. Packaging. Solid waste will be placed in cans, sturdy bags, or boxes.Rigid, puncture-resistant, sealable containers will be used for packaging "sharps." When wet materials are packaged for disposal, the materials will be placed in a leak-proof container. Heavy waste will be placed in rigid containers ensuring that the burst strength of the container is not exceeded.

g. Labeling. A method of verifying that all items prepared for disposal have been decontaminated will be established for etiologic agent wastes. Mixed waste will be labeled as appropriate to indicate the hazards that must be addressed after decontamination.

h. Recordkeeping. A manifest will be initiated and maintained, where required, to record the disposition and transfer of waste.Applicable Federal, State, and local ordinances will be followed.

Chapter 6
Importation, Shipment, and Transport of Etiologic Agents

6–1. Introduction
The CDC of the Public Health Service (PHS), the U. S.Department of Agriculture (USDA), the Food and Drug Administration(FDA), the Department of Transportation (DOT), the U.S. Postal Service, and the International Air Transport Association (IATA)regulate the importation, shipment, and transportation of etiologic agents. This chapter outlines the minimum administrative requirements the commanders or institute directors are to follow and gives sources for information on the requirements for importation, packaging, labeling, and shipment of etiologic agents.

6–2. Administration
The commander or institute director will establish the following controls to ensure that etiologic agents are transported with proper authorization, controls, and procedures:

a. Institute policies will be established in writing to ensure that before etiologic agents are acquired or shipped—

(1) The division chief overseeing the area where work with etiologic agents is to be conducted approves all acquisitions or shipments.

(2) The safety officer is informed in writing of the type and amount of any BL–4 or USDA restricted etiologic agent (listed in HHS publication No. (NIH) 88–8395 or current edition) being received, and the estimated date of arrival.

(3) The recipient of all etiologic agents shipped from an institute will be documented.

(4) The commander or institute director approves all acquisitions and shipments of BL–4 or USDA restricted etiologic agents.

(5) The commander or institute director approves all requests for shipments to or from foreign countries and to individuals not affiliated with an institution or agency (for example, physicians in private practice).

(6) The Office of The Surgeon General, U.S. Army, or the Commander, U.S. Army Materiel Command (AMC) approves the initial acquisition and use of all reference stocks of etiologic agents and transfers between Army RDTE activities in accordance with AR 70–65.

(7) There is full compliance with the regulatory requirements referenced in paragraphs 6–3, 6–4, 6–5, and 6–6.

(8) The following information regarding the recipient and the intended use of BL–4 and USDA restricted animal pathogens, will be kept on file for 10 years. This information will also be kept for all shipments to or from foreign countries and to individuals not affiliated with an institution or agency (for example, physicians in private practice).

(a) The requester's name and address.

(b) The type and amount of the etiologic agent to be sent.

(c) The qualifications of the recipient of the etiologic agent.

(d) The intended use of the etiologic agent.

(e) A statement indicating that the agent is not for human use.

b. Etiologic agents assigned to biosafety levels 1, 2, or 3, approved for shipment, and properly labeled and packaged may be shipped by commercial cargo carriers.

c. All etiologic agents assigned to BL–4 or USDA restricted animal pathogens approved for shipment and properly packaged, will be accompanied by a designated courier, or under close supervision of a responsible party who will monitor aspects of the shipment, ensuring that required transfers have been completed and documented and final receipt has been accomplished and acknowledged.

6–3. Importation directives
Importation of etiologic agents is subject to the Public Health Service Foreign Quarantine Regulations (42 CFR 71.156).Examples of permits authorizing the importation or receipt of regulated materials and specifying conditions under which the etiologic agent is shipped, handled, and used are contained in paragraph 6–4.

6–4. Shipment directives
Shipping unmarked and unidentified etiologic agents is prohibited. Etiologic agents will be packaged, labeled, and shipped according to the requirements found in the Interstate Shipment of Etiologic Agents Regulations (42 CFR 72) and its amendments. The USDA regulations in 9 CFR 102 through 104, 122 and the FDA regulations in 21 CFR 312 and 600 through 680 will also be followed as applicable. Packaging and labeling requirements for interstate shipment of etiologic agents are contained in HHS publication No.(NIH) 88-8395. Guidelines for the Air Shipment of Diagnostic Specimens may be obtained from the Air Transport Association of America, Cargo Services Division, 1709 New York Ave. NW., Washington, DC 20006. All etiologic agents used in the BDP will be shipped using secondary shipping containers sealed with crimped lids. The following documents contain the requirements which must be met when shipping regulated materials and specify the conditions under which an etiologic agent may be shipped, handled, and used:

a. Permit Application to Import or Transport Agents or Vectors of Human Disease published by the Department of Health and Human Services, PHS, CDC, Office of Biosafety, Atlanta, Georgia 30333.

b. Permit Application to Import Controlled Material; Import or Transport Organisms or Vectors published by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Federal Building, Hyattsville, Maryland 20782.

6–5. Transportation directives
The packaging and labeling requirements cited above must be followed for the local transport of etiologic agents and diagnostic specimens by courier or by other delivery services. Similar requirements and restrictions applicable to the transport of etiologic agents,

diagnostic specimens, and biological products by all modes of transportation (that is, air, motor, rail, and water)are imposed by the Department of Transportation (49 CFR 173), IATA"Dangerous Goods Regulations," the Air Transport Association "Restricted Articles Tariff 6–D,"the International Civil Aviation Organization (ICAO), Postal Bulletin No. 21246 "International Mail-Hazardous Materials," 39 CFR, and, the Domestic Mail Manual. When shipments exceed 4 liters, the requirements found in AR 740–32 will be followed.

6–6. Additional requirements

Additional requirements for importation, shipment, and transportation of infectious agents and hazardous materials that must be followed are contained in AR 40–12 and AR 70–65.

6–7. Sources for further information on shipment of etiologic agents

a. Guide for Transportation of Hazardous Materials, Vol. 4(1).February 10, 1975. Copies are obtainable from the Office of Research Grants Inquiries, NIH, Department of Health and Human Services, 5333 Westbard Avenue, Bethesda, MD 20205.

b. The CDC, Office of Biosafety, 1600 Clifton Road NE., Atlanta, Georgia 30333. Telephone (404) 639-3883, or FTS: 236-3883.

c. The American Type Culture Collection (ATCC), Packaging and Shipping of Biological Materials at ATCC. Copies may be obtained from the ATCC, 12301 Parklawn Drive, Rockville, MD 20852. Phone(301) 881-2600.

d. National Committee for Clinical Laboratory Standards(NCCLS), Procedures for the Domestic Handling and Transport of Diagnostic Specimens and Etiologic Agents. (H5–A2), Second edition. Vol. 5, No. 1. Copies are obtainable from the NCCLS, 771 East Lancaster Avenue, Villanova, PA 19085.

Chapter 7
Facilities

7–1. Introduction

The design of the facility is important in providing a secondary barrier to protect individuals inside and outside the facility. Because the hazards presented by various organisms and materials vary, the requirements for the facility will vary accordingly. The minimum facility requirements for the various biosafety levels and toxins are described below. The biosafety levels correspond to those described in the HHS Publication Biosafety in Microbiological and Biomedical Laboratories (HHS No.(NIH) 88–8395), while the large-scale biosafety levels were adapted from those described in the NIH Guidelines for Research Involving Recombinant DNA Molecules.

7–2. Biosafety level 1

a. Laboratories. Each laboratory used for this level will, as a minimum, have the following features:

(1) A sink for handwashing.

(2) Work surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

(3) Fly screens on any windows that can be opened.

(4) Furnishings and surfaces that are sturdy and designed to be easily cleaned.

(5) Spaces between furnishings and equipment that are accessible for cleaning.

b. Animal facilities. Each room will have the following features:

(1) Design and construction to facilitate cleaning and housekeeping.

(2) A sink for handwashing within the facility.

(3) Fly screens on any windows that can be opened.

(4) Ventilation designed so that the direction of airflow in the animal facility is inward, with the exhausted air discharged to the outside without being recirculated.

(5) Self closing doors that open inward.

7–3. Biosafety level 2

a. Laboratories. Each laboratory used for this level of hazard will have, in addition to the requirements stated in paragraph 7–2 *a* above, the following:

(1) An autoclave available.

(2) Containment equipment necessary for the operations unless the safety officer approves the use of a compensatory level of personal protective equipment.

(3) An eyewash available near the laboratory.

b. Animal facilities. In addition to the requirements stated in paragraph 7–2 *b* above, facilities will include—

(1) A sink for handwashing in each room where animals are housed.

(2) An autoclave available in the building.

(3) Appropriate containment equipment unless the safety officer approves the use of a compensatory level of personal protective equipment.

7–4. Biosafety level 3

a. General requirements. Each suite used as a laboratory or in which infected animals are housed will, as a minimum, have the following features:

(1) Physical separation from areas which are open to unrestricted traffic.

(2) All entrances to each laboratory or animal room from the nonlaboratory access corridors will be through two sets of doors. A change room or airlock may be incorporated between the doors.

(3) The interior surfaces of walls, floors, and ceilings will be water resistant so that they may be easily cleaned.

(4) All penetrations into the walls, floors, and ceilings should be sealed or capable of being sealed to facilitate decontamination.

(5) A foot, elbow, or automatically operated sink will be located near the exit door to each laboratory or animal room.

(6) An autoclave should be in each laboratory or animal room and will be available to the facility.

(7) A ventilation system that will—

(a) Create directional airflow that draws air into the laboratory through the entry areas.

(b) Not recirculate laboratory air.

(c) Discharge the exhaust air from the laboratory to the outside and disperse the exhaust air away from occupied areas and air intakes.

(d) Exhaust the HEPA filtered air from Class I or II biological safety cabinets or other primary containment devices directly to the exterior of the laboratory or through the building exhaust system.Exhaust air from the cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every 12 months. If the filtered cabinet exhaust is discharged through the building exhaust system, it will be connected to this system in a manner (for example, thimble unit connection) that avoids any interference with the air balance of the cabinets or the building exhaust system.

(8) All windows to the facility will be sealed shut.

(9) Appropriate biological safety cabinets or other specialized containment equipment will be provided.

(10) Any vacuum line in the facility will have a HEPA filter and liquid disinfectant trap.

(11) Bench tops that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

(12) Furnishings that are sturdy and spaces between benches, cabinets, and equipment that are accessible for cleaning.

(13) An eyewash available in or near the laboratory.

b. Additional animal facility requirements. In addition to the requirements given in paragraphs 7-–3 *b* and 7–4 *a*above, all doors to the animal rooms will open inward and be self-closing.

7–5. Biosafety level 4

The engineering controls within the facility must provide absolute biological containment. All procedures with etiologic agents requiring this biosafety level of facilities, equipment, and procedures must be conducted either in Class III biological safety cabinets, or in a facility that is designed for the use of a personal positive pressure

suit as described in b below in conjunction with Class I or II biological safety cabinets.

a. General requirements. The facility will have the following features:

(1) A separate building or a clearly demarcated and isolated area within a building which incorporates positive personnel control for access.

(2) All entrances from access corridors incorporate an inner and outer change room.

(3) Inner and outer change rooms separated by a shower facility.

(4) A double doored autoclave, fumigation chamber, or ventilated airlock for passage of all items which do not enter the facility through the change room.

(5) Interior surfaces of walls, floors, and ceilings resistant to water and chemicals to facilitate cleaning and disinfecting.

(6) Walls, floors, and ceilings of the facility constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof.

(7) All penetrations into the walls, floors, and ceilings sealed.

(8) All liquid drains in the facility connected directly to a liquid waste decontamination system.

(a) Holding tanks collecting waste from sinks, biological safety cabinets, floors, and autoclave chambers provide decontamination by heat treatment.

(b) Holding tanks collecting waste from shower rooms and toilets provide decontamination by heat or chemical disinfectant methods.

(9) Sewer and other ventilation vents contain in-line HEPA filters.

(10) Internal facility appurtenances (for example, light fixtures, air ducts, and utility pipes) arranged to minimize the horizontal surface area on which dust can settle.

(11) A foot, elbow, or automatically operated handwashing sink located near the exit door to each laboratory or animal room.

(12) Self closing and lockable access doors.

(13) A ventilation system that—

(a) Is dedicated to the facility and provides fresh air meeting American Society of Heating, Refrigerating, and Air Condition Engineers, Inc. (ASHRAE) Standard 62.

(b) Maintains a negative pressure differential and assures flow inward from areas outside of the facility toward areas of highest potential risk.

(c) Has manometers or magnehelic gauges to provide, sense, and display pressure differentials between adjacent areas maintained at different pressure levels. An alarm will sound when the pressures fall below acceptable levels.

(d) Has the air supply and exhaust interlocked to ensure that exhaust failure or reduction will not allow the air pressure in the area to become positive to the adjacent areas.

(e) Does not recirculate exhaust air.

(f) Is HEPA filtered and discharged to the outside, dispersing the exhaust air away from occupied areas and air intakes.

(g) Has the HEPA filters on the exhaust located as near to the rooms as is practicable.

(h) Has the filter chambers designed to allow in-place decontamination before the filters are removed and to facilitate certification testing.

(i) Contains prefilters and HEPA filters in the air supply system to protect the supply air system should air pressures become unbalanced.

(j) Exhausts the HEPA filtered air from Class I or II biological safety cabinets directly into the laboratory or to the exterior of the building. If the HEPA filtered exhaust from these cabinets is recirculated, the cabinets are tested and certified every 6 months. If the filtered cabinet exhaust is discharged through the building exhaust system, it will be connected to this system in a manner (for example, thimble unit connection) that avoids any interference with the air balance of the cabinets or the building exhaust system.

(k) Passes the treated exhaust air from Class III biological safety cabinets through two sets of HEPA filters in series to the exterior of the facility through the laboratory exhaust air system.

(14) Windows (if present) sealed shut and breakage resistant.

(15) A double doored autoclave for decontaminating materials passing out of the facility. The autoclave door that opens to the area external to the facility is sealed to the outer wall and automatically controlled so that it can only be opened after the autoclave sterilization cycle has been completed.

(16) A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method for materials and equipment that cannot be autoclaved.

(17) Central vacuum systems (if present) that—

(a) Do not serve areas outside the facility.

(b) Have an in-line HEPA filter placed as near as practicable to each use point or service cock.

(c) Have filters designed to allow in-place decontamination and replacement.

(18) Liquid and gas services to the facility provided with protective devices that prevent backflow.

b. Additional requirements for personal positive pressure suit areas. If personal positive pressure suits are worn instead of using Class III biological safety cabinets for containment, a special suit area will be provided. The suit area will provide the following, in addition to the requirements stated in a above:

(1) An exhaust system dedicated to that area that provides filtration by two sets of HEPA filters installed in series. This system will be backed up by a duplicate filtration unit, exhaust fan, and an automatically starting emergency power source. The ventilation system will maintain the suit area under negative pressure relative to the surrounding areas.

(2) An entry area consisting of an airlock fitted with airtight doors.

(3) A chemical shower to decontaminate the surface of the personal positive pressure suit upon exit.

(4) An air supply and distribution system to support the life support system of the personal positive pressure suits.

(5) Emergency lighting and communications systems.

(6) Sealed penetrations into the internal shell of the area.

(7) A double doored autoclave to decontaminate waste materials to be removed from the suit area.

c. Additional laboratory requirements. In addition to those given in paragraph 7–4 above, if water fountains are provided, they will be foot operated and located in the facility corridors outside the laboratory.

d. Additional animal facility requirements. In addition to those requirements given in paragraph 7–4 above, all animal facility external doors will be self locking.

7–6. Large-scale facilities

The following requirements apply to facilities in which an individual culture of viable etiologic agents exceeds 10 liters:

a. BL–1 LS. In addition to the laboratory requirements stated in paragraph 7–2 *a*, the exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (for example, incineration) to minimize the release of viable organisms.

b. BL–2 LS. In addition to the requirements stated in paragraphs 7–3 *a* and *a* above, these facilities will have—

(1) Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms will be designed to prevent leakage or will be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to HEPA filters or through equivalent treatment devices.

(2) A closed system used to propagate and grow viable organisms will include monitoring or sensing devices that monitor the integrity of containment during operations.

(3) Closed systems used for the propagation and growth of viable organisms will be tested operationally for integrity of the containment features. The containment will be rechecked following modification or replacement of essential containment features.Procedures

and methods used in the testing will be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results will be maintained on file.

c. BL–3 LS. The requirements stated in paragraphs 7–4 and *b* above apply, and all closed systems and other primary containment equipment used in handling cultures of viable organisms will be located within a controlled area which meets the requirements of a BL–3 facility plus the following requirements:

(1) All utilities and service or process piping or wiring entering the controlled area will be protected against contamination.

(2) A shower facility will be provided. This facility will be located near the controlled area.

(3) The controlled area will be designed to preclude release of culture fluids outside in the event of an accidental spill or release from the closed systems or other primary containment equipment.

(4) The controlled area will have a ventilation system capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure supply air, the system shall operate so as to prevent the reversal of air movement or shall be equipped with an alarm that would be actuated if reversal in the direction of air movement were to occur.The exhaust air from the controlled area shall not be recirculated to other areas of the facility. The exhaust air from the controlled area may be discharged to the outdoors after filtration or other means of effectively reducing an accidental aerosol burden and dispersed clear of occupied buildings and air intakes.

7–7. Toxins

a. All facilities in which toxins are used will—

(1) Have a ventilation system that provides three to six air changes per hour, and that provides a directional airflow inward relative to the access halls.

(2) Have a sink for handwashing.

(3) Have an eyewash available.

(4) Have bench tops that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

(5) Have furniture, furnishings, and surfaces that are sturdy and designed to be easily cleaned.

(6) Be arranged so that items are accessible for cleaning.

(7) Have a quick-drench shower available within the facility.

(8) Have a fume hood, biological safety cabinet, glove box, or equivalent engineering control equipped with HEPA filters and with charcoal filters if volatile materials are being used.

b. Not used.

Chapter 8
Engineering Controls

8–1. Introduction

As required by the OSHA and recommended by the American Industrial Hygiene Association (AIHA) and the CDC, engineering controls and proper microbiological techniques are the primary means of protecting personnel who work with potentially hazardous biological materials. In situations of potentially higher hazard, these engineering controls are supplemented by personal protective clothing and equipment. Thus, the engineering controls discussed in this chapter will be the primary means of personnel and environmental protection when working with etiologic agents. Because of the importance of these engineering controls, this chapter contains not only requirements for the engineering and construction of these controls, but also requirements for their certification and continuous satisfactory performance. These will be described for each engineering control.

8–2. Class I biological safety cabinets

a. Description. The Class I biological safety cabinet (fig 8–1) is a ventilated cabinet for personnel protection only. The cabinet provides an uncirculated inward flow of air away from the operator.The exhaust is passed through a HEPA filter. It may be discharged into the laboratory or vented out of the laboratory and dispersed away from occupied spaces or air intakes. When the exhaust is recirculated in a BL–2 or BL–3 facility, the cabinet must be tested and certified annually. In a BL–4 facility, if the exhaust is recirculated, the cabinet must be tested and certified semiannually.

b. Uses. These cabinets are used if personnel protection against the microorganisms is required; for modest quantities of volatile, toxic, or radioactive chemicals (in concentrations and quantities associated with biological systems) if vented to the outside; and when sterility is not required. They are commonly used for housing tabletop centrifuges, in the necropsy of small animals, and for changing animal bedding.

c. Prohibitions. This class of cabinet is not to be used when sterility must be maintained. In addition, volatile, toxic, or radioactive materials can not be used in this class of cabinet when the exhaust air is not exhausted to the exterior.

d. Certifications and requirements.

(1) The inward air velocity on these cabinets will be an average of 100 plus or minus 20 linear feet per minute (lfpm). Each cabinet must be certified before use and semiannually thereafter by a face velocity test. Additionally, smoke tests will be performed annually to verify containment.

(2) The exhaust system will have a HEPA filter, which will be tested initially upon installation, after repair or replacement, and every 2 years thereafter (except when required more often). Filters will be certified to be 99.97 percent effective in capturing particulate matter by a leakage test using mineral oil or other appropriate aerosol dispersed as 0.3 micron droplets.

8–3. Class II biological safety cabinets

All Class II biological safety cabinets (fig 8–2) are ventilated cabinets for personnel and product protection, having an open front with inward air flow for personnel protection.

a. Operating standards. All of these cabinets must conform and be certified to meet National Sanitation Foundation (NSF) Standard No. 49 revised, June 1987, for the applicable type of cabinet. After installation and before use, and annually thereafter, the cabinets will be tested according to NSF Standard No. 49 (latest revision, June 1987) as follows:

(1) Primary (required) tests are as follows:

(a) Velocity profile test.

(b) Work access opening airflow (face velocity) test.

(c) HEPA filter leak test.

(d) Cabinet integrity test (soap bubble test) for cabinets with positive pressure internal plenums.

(2) Secondary (optional) tests are as follows:

(a) Vibration test.

(b) Electrical leakage and ground circuit resistance tests.

(c) Noise level test.

(d) Lighting intensity test.

(e) UV light intensity test.

(3) After repairs or alterations to the cabinetry or ventilation system that affect the cabinet, the tests listed in *a* (2) above will be performed for the relevant parameters.

(4) The work access opening airflow (face velocity) test, as specified in NSF Standard No. 49 (latest revision, June 1987), will be performed to check that the cabinet is within specifications on an annual basis for BL–1 and BL–2 and toxin use. This test will be performed semiannually on cabinets used for BL–3 and BL–4 as well as for work with dry forms of toxins.

(5) When the exhaust is recirculated in a BL–4 facility, the cabinet must be tested and certified semiannually.

b. Class II, Type A biological safety cabinets.

(1) Description. A Class II, Type A biological safety cabinet is one in which typically 70 percent of the air is recirculated within the cabinet and the exhaust passes through a HEPA filter before discharge. The exhaust may be exhausted into the room and positive-pressure contaminated ducts and plenums within the cabinet are

allowed. Type A cabinets will have a minimum calculated face velocity of 75 feet per minute (fpm).

(2) Uses. These cabinets are for working with low-to-moderate risk biological samples and for protecting personnel against biological material while providing a sterile atmosphere in which to handle the material.

(3) Prohibitions. Materials that are toxic or volatile must not be used in these cabinets.

c. Class II, Type B1 biological safety cabinets.

(1) Description. A Class II, Type B1 biological safety cabinet is one that maintains a minimum average inflow of air of 100 plus or minus 20 lfpm and in which typically 30 percent of the air is recirculated. All recirculated and exhausted air passes through two HEPA filters in series. All contaminated internal ducts and plenums are under negative pressure. Type B cabinets will have a minimum calculated face velocity of 100 fpm.

(2) Uses. When ultra-sterility is needed, these are the cabinets of choice. The double filtration achieves a cleaner atmosphere. Minute quantities of volatile, toxic, or volatile radioactive materials coincidental to use in biological systems may also be used in these cabinets.

(3) Prohibitions. More than minute quantities of toxic, volatile, or radioactive materials must not be used in these cabinets.

(4) Additional certifications or requirements. None exist.

d. Class II, Type B2 biological safety cabinets.

(1) Description. A Class II, Type B2 biological safety cabinet is one that maintains a minimum average of 100 plus or minus 20 lfpm inward flow and in which all air is exhausted directly from the cabinet through a HEPA filter without recirculation within the cabinet. All contaminated ducts and plenums are under negative pressure. Type B cabinets will have a minimum calculated face velocity of 100 fpm.

(2) Uses. These cabinets are recommended when small quantities of volatile, flammable, or toxic chemicals must be used coincidentally with items requiring sterility.

(3) Prohibitions. While these cabinets do offer the greatest degree of safety for volatile, toxic, and flammable chemical handling in a sterile environment, they are not to be used in place of a fume hood to prepare stock solutions of hazardous chemicals.

e. Class II, Type B3 biological safety cabinets.

(1) Description. A Class II, Type B3 biological safety cabinet is one that meets all of the requirements of a Class II, Type B2 biological safety cabinet except that it recirculates most(typically 70 percent) of the air inside the cabinet. A Type B cabinet will have a minimum calculated face velocity of 100 fpm. It must also meet all the requirements of a Class II, Type A cabinet if used as such.

(2) Uses. Minute amounts of nonflammable chemicals can be used coincidentally with low-to-moderate risk biological agents.

(3) Prohibitions. Flammable materials and more than minute amounts of toxic, radioactive, or volatile chemicals must not be used in these cabinets.

(4) Additional certifications or requirements. None exist.

8–4. Class III biological safety cabinets

a. Description. These cabinets (fig 8–3) are totally enclosed, ventilated cabinets of gas-tight construction. Operations are conducted through attached rubber gloves. The supply of air is drawn into the cabinet through HEPA filters. The exhaust air is treated by double HEPA filtration, or by HEPA filtration followed by incineration, and is not allowed to recirculate within the room.

b. Uses. These cabinets provide the ultimate protection for personnel. They are suitable for low, moderate, and high-risk etiologic agents.

c. Prohibitions. More than minute amounts of flammables must not be used in these cabinets.

d. Certifications and requirements.

(1) These cabinets will have a manometer or magnehelic gauge that indicates the negative pressure that is maintained inside the cabinet. The pressure inside the cabinet should be a minimum of 0.5 inches water gauge negative to the surrounding room.

(2) These cabinets will be pressure tested by the soap bubble or halogen leak test as prescribed in NSF Standard No. 49, appendix B1 (latest revision, June 1987), and certified, when the HEPA filter units are serviced.

8–5. Fume hood

Fume hoods in which etiologic agents are handled must use proven technologies to provide optimal containment. Fume hood placement, design, and capture testing requirements for use in designing new laboratories can be found in the latest edition of Industrial Ventilation, A Manual of Recommended Practice, published by the American Conference of Governmental Industrial Hygienists.

a. Description. Fume hoods are common chemical laboratory furnishings designed to capture fumes from chemicals that are used within them.Air is drawn through the opening and vented to the exterior without recirculation.

b. Uses. Fume hoods provide excellent containment for handling hazardous chemicals.

c. Prohibitions. Moderate risk biologicals and open containers of dry forms of toxins must not be used in a fume hood without HEPA filtration.Fume hoods should never be used when sterility is required.

d. Certifications and requirements.

(1) Inward air flow will be an average of 100 lfpm (150 lfpm for carcinogens) plus or minus 20 lfpm as measured at the face of the fume hood. Proper function of laboratory hoods is not only a function of face velocity. An evaluation of the total operating environment is necessary.

(2) When filters are required, they will be certified by the mineral oil droplet (HEPA) or Freon (charcoal) leak test as appropriate. Leakage through the filters will be less than 0.05 percent for Freon and 0.03 percent for oil droplets when initially installed.

(3) Fume hoods will be provided with indicator devices to give a warning should the ventilation system fail or if the hood face velocity falls below an average of 80 lfpm.

(4) Hood air flow will be certified when installed, when maintenance is performed on the ventilation system, and semiannually thereafter.

8–6. Glove box

a. Description. A glove box is an enclosure that provides a positive barrier from liquids, solids, and chemical vapors. A glove box has viewing ports and glove ports for access. The box maintains personnel protection through solid barriers and maintenance of a negative pressure relative to its surroundings.

b. Uses. Glove boxes are used when extreme containment is needed for highly toxic chemicals, especially for dry chemicals that can be swept out of containers by the airflow in hoods.

c. Prohibitions. Unventilated boxes must not be used with volatile flammable materials and should not be used with volatile toxic materials unless dilution ventilation is provided.

d. Additional certifications and requirements.

(1) The glove box will be maintained at a pressure of at least 0.25 inches water gauge less than its surroundings.

(2) The pressure differential will be indicated by a manometer or magnehelic gauge. Indicator devices will display a loss of pressure below 0.25 inches water gauge.

(3) Gloves will be changed at appropriate intervals (dependent on the box contents) to ensure they provide the protection needed.

(4) Inlets that provide dilution air will be protected by HEPA filters.

8–7. Ventilated balance enclosures

a. Description. A ventilated balance enclosure is a box that surrounds a balance and has a small open area for access and handling material in the front. Air is exhausted out the rear of the enclosure.

b. Uses. A ventilated balance enclosure is used when containment of a balance is required to weigh hazardous materials that have a low vapor pressure (such as toxins). These enclosures are also used when it is best to use the balance in other than a fume hood (due to the turbulence and vibration) and when biological safety

cabinets or glove boxes are inappropriate or unavailable. Dry forms of toxins may be weighed in these enclosures.

c. Prohibitions. Very volatile or highly toxic volatile materials must not be handled in ventilated balance enclosures unless they are placed in closed containers in a properly functioning fume hood before being transferred to the balance enclosure.

d. Additional certifications or requirements.

(1) The flow through the openings in the enclosure will be at least 60 lfpm and must average between 60 and 80 lfpm.

(2) Containment will be certified prior to first use and annually thereafter by smoke tubes.

(3) The air flow will be certified initially and semiannually by averaging readings taken from the face of the opening.

8–8. Ventilated cage enclosures

There are a number of cage ventilated enclosures in which infected animals may be housed at levels corresponding to the various classes of biological safety cabinets. A brief description of four different types of ventilated animal cages is given below.This is not a complete description of all the different ventilated animal cages available. The proper functioning of these will be tested initially, upon each connection to exhaust sources, and at least annually. The inward flow rates on the partial containment systems and pressure checks on the total containment cages will be performed. Prior to selecting such equipment, an evaluation of the function and the equipment should be made, and the methods for testing and decontamination should be analyzed and documented.

a. Filter-top cages. Small laboratory animal polystyrene or polycarbonate cage bottoms are fitted with a dome shaped glass fiber or polyester filter cage cover. The dome shaped filters help reduce the dissemination of aerosols, and the spread of infectious agents.Adequate ventilation around cages fitted with a dome shaped filter is essential since they may contain elevated ammonia and carbon dioxide levels, and high temperature and humidity. Ventilation recommendations in the NIH publication 86–23, 1985 'Guide for the Care and Use of Laboratory Animals' will be followed.

b. Forced ventilation cages. This is a small HEPA filtered animal cage connected to a centralized exhaust system. A minimum airflow of 0.03 m3/min per cage is required. Ventilation rates may vary with the size of the cage, and the number and type of animals being housed.

c. Cubicle-type isolation cage. This is a partial containment unit which holds several animal cages. This unit is a negative pressure HEPA filtered stainless steel cage. A minimum airflow of 0.3 m3/min per cage is required for a 0.24 m3 unit. Ventilation rates may vary with the size of the cage and the number and type of animals being housed.

d. Total containment cage. This unit is a negative pressure or positive pressure HEPA filtered stainless steel cage which has the filters incorporated into the design. It is halogen gas-leak tight and can be considered a Class III biological safety cabinet. A minimum airflow of 0.3 m3/min per cage is required for a 0.24 m3 unit. Ventilation rates may vary with the size of the cage, and the number and type of animals being housed.

8–9. Ventilated cage areas

Ventilated cage areas are areas within a room that are solid-walled and bottomed areas for containing multiple cages housing infected animals. The containment for these areas is equivalent to the Class I biological safety cabinet. For testing purposes, they will be treated the same as a Class I biological safety cabinet. Smoke tests will be performed annually to verify containment.

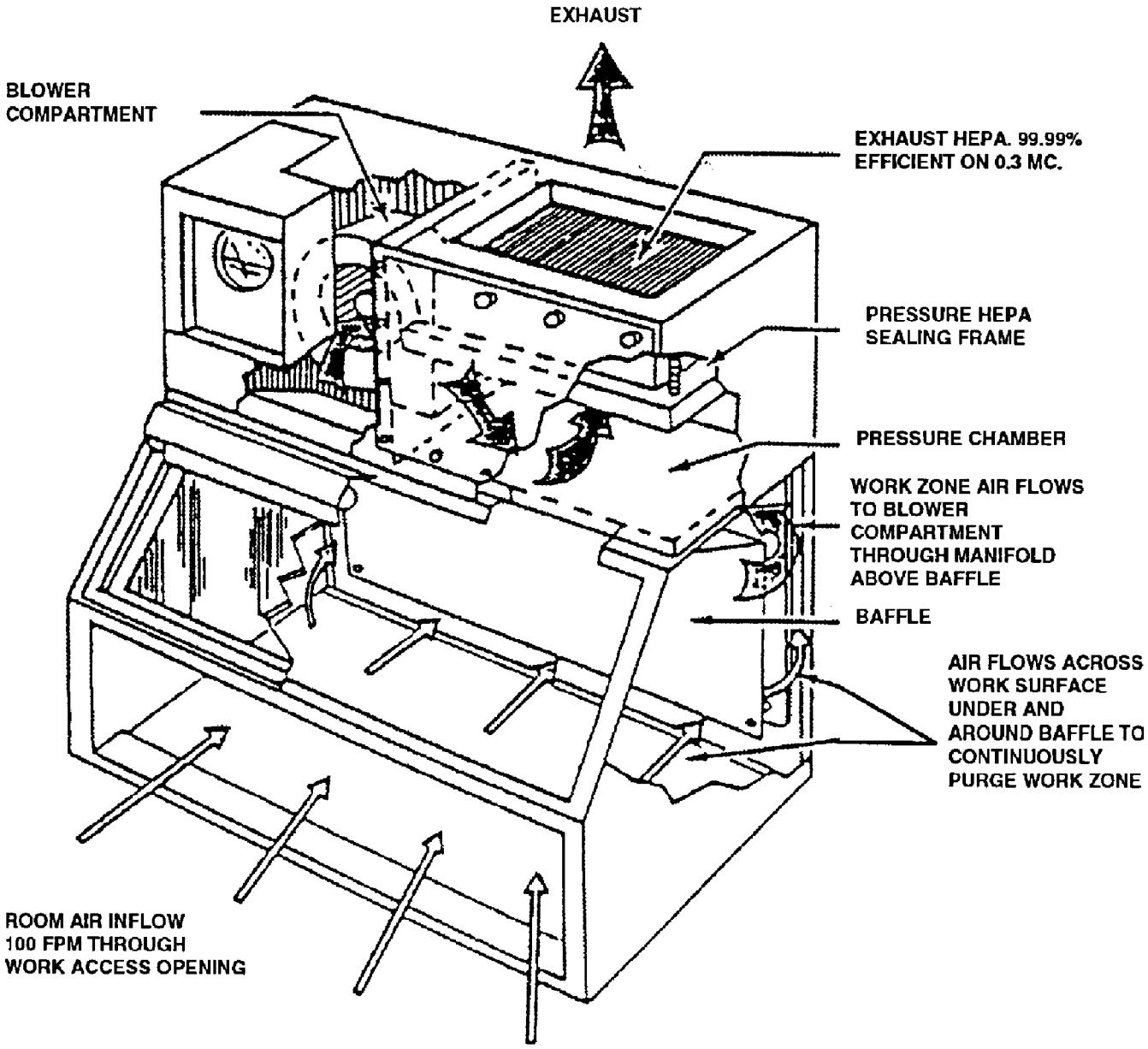

Figure 8-1. Class I Biological Safety Cabinet

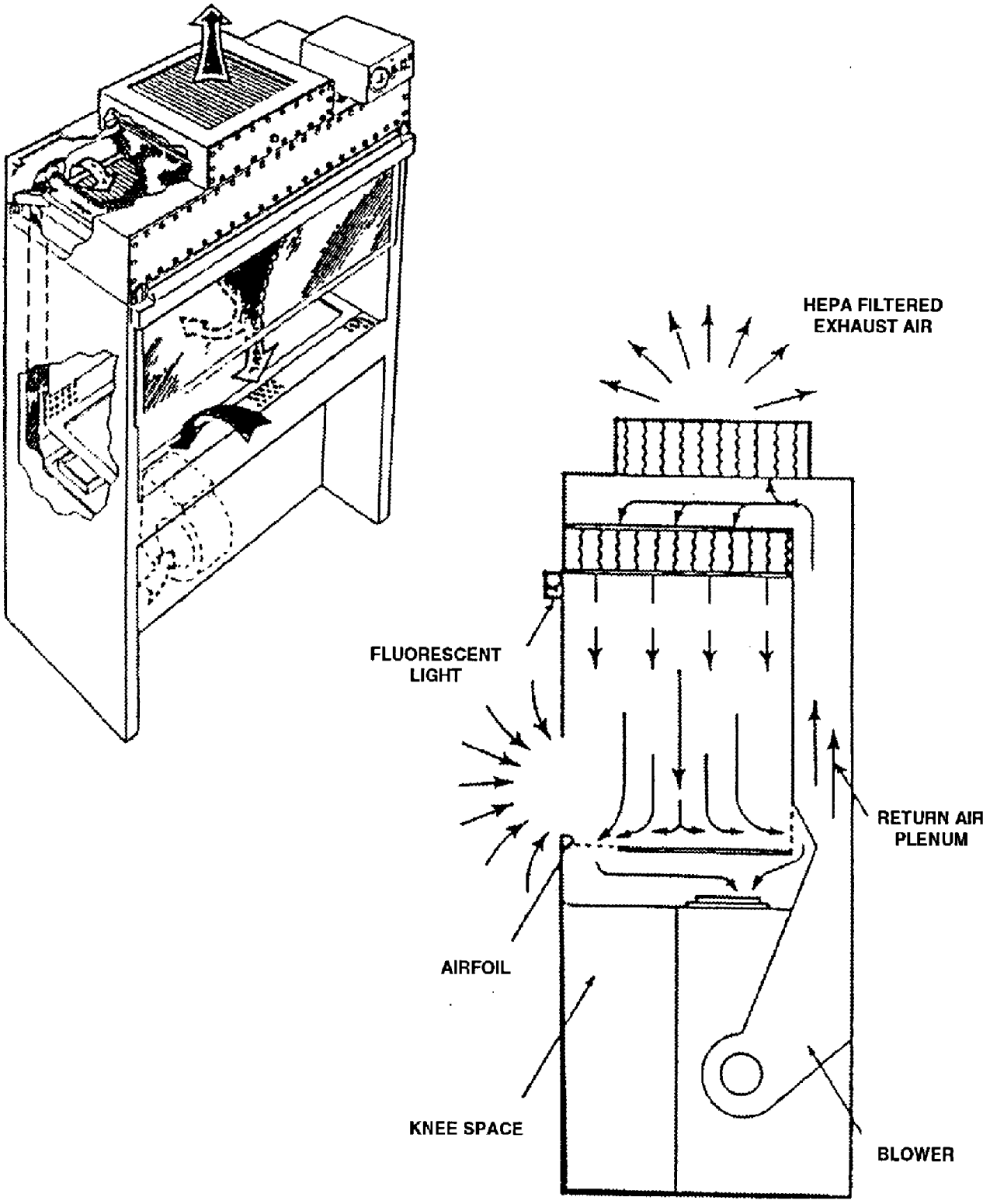

Figure 8-2. Class II Biological Safety Cabinet

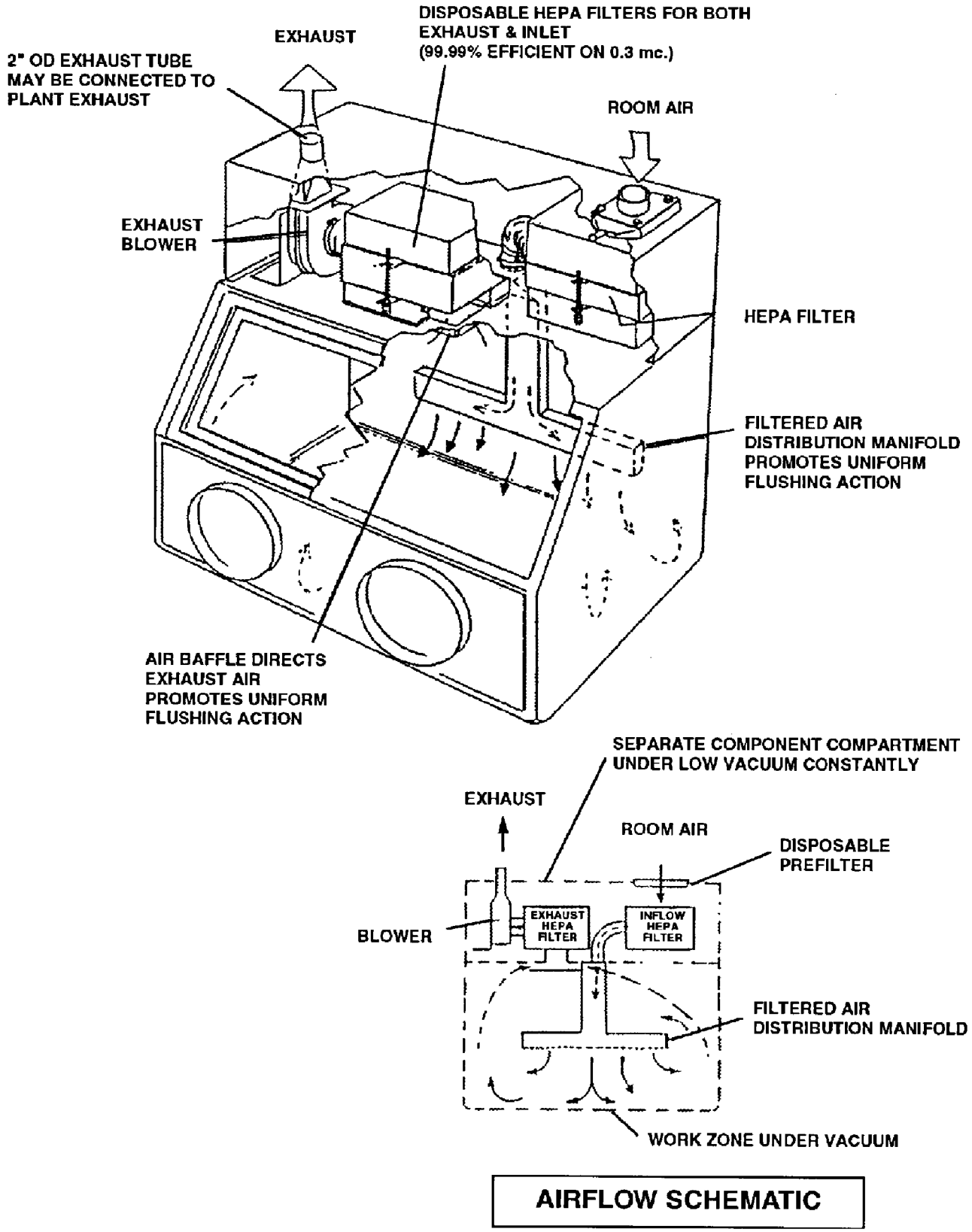

Figure 8-3. Class III Biological Safety Cabinet

Appendix A
References

Section I
Required Publications

AR 11–34
The Army Respiratory Protection Program. (Cited in para 4–11 *h*(2) and (4).)

AR 40–5
Preventive Medicine. (Cited in para 2–3.)

AR 40–10
Health Hazard Assessment Program in Support of the Army Materiel Acquisition Decision Process. (Cited in para 2–2 *a*(8).)

AR 40–12
Quarantine Regulations of the Armed Forces. (Cited in para 6–6 *a*.)

AR 40–66
Medical Record and Assurance Administration. (Cited in para 2–4.)

AR 40–400
Patient Administration. (Cited in para 2–3 *e*.)

AR 70–65
Management of Controlled Substances, Ethyl Alcohol, and Hazardous Biological Substances in Army Research, Development, Test, and Evaluation Facilities. (Cited in paras 6–2 *a*(6) and 6–6 *b*.)

AR 385–10
Army Safety Program. (Cited in paras 2–1 and 4–11 *h*(4).)

AR 385–11
Ionizing Radiation Protection(Licensing, Control, Transportation, Disposal, and Radiation Safety). (Cited in para 3–ll *a*.)

AR 385–69
Biological Defense Safety Program.(Cited in paras 2–1, 2–2 *a*, 2–2 *a*(8), 2–2 *d*, 3–2 *c*, 3–9 *a* and *f*(1).)

AR 740–32
Responsibilities for Technical Escort of Dangerous Materials. (Cited in para 6–5.)

Section II
Related Publications

A related publication is merely a source of additional information. The user does not have to read it to understand this regulation.

AR 40–14
Control and Recording Procedures for Exposure to Ionizing Radiation and Radioactive Materials

Acceptance of Hazardous, Restricted, or Perishable Matter Publication 52, April 1990 (This publication is available from the U.S. Postal Service, Washington, DC.)

Industrial Ventilation|A Manual of Recommended Practice, 17th Ed., 1982. (This publication is available from the American Conference of Governmental Industrial Hygienists, Cincinnati, OH.)

ASHRAE Standard 62-1981, Ventilation for Acceptable Indoor Air Quality (This publication is available from the American Society of Heating, Refrigerating, and Air Conditioning Engineers, Inc., Atlanta, GA.)

Bacterial Toxins: A Table of Lethal Amounts, Microbiological Reviews, Volume 46, Number 1; March 1982, pages 86-94 (This publication is available from the American Society for Microbiology Publications Office, 1913 I Street N.W., Washington DC 20006.)

Biohazards Reference Manual, 1985 (This publication is available from the American Industrial Hygiene Association, Akron, OH.)

Commodity Specification for Air Compressed Gas Association Pamphlet G-7.1, 1989, and American National Standard ANSI Z86.1, 1973 (This publication is available from the Compressed Gas Association, Arlington, VA.)

Dangerous Goods Regulations (This publication is available from the International Air Transport Association (IATA), Publications Section, 2000 Peel Street, Montreal, Quebec, Canada H3A 2R4, Tel (514) 844–6311.)

Guidelines for Research Involving Recombinant DNA Molecules; Notice. Federal Register, Vol 51, No. 88,16958–16985, May 7, 1986

Guidelines for Research Involving Recombinant DNA Molecules Actions Under Guidelines; Notice. Federal Register, Vol 52, No. 163, 31848–31850, August 24, 1987

Guidelines for Research Involving Recombinant DNA Molecules Actions Under Guidelines; Notice. Federal Register, Vol 53, No. 146, 28819, July 29, 1988

Guidelines for Research Involving Recombinant DNA Molecules Actions Under Guidelines; Notice. Federal Register, Vol 53, No. 207, 43410–43411, October 26, 1988

Recombinant DNA Research; Actions Under Guidelines; Notice. Federal Register, Vol 54, No. 47,10508–10510, March 13, 1989

DHEW Pub. No. (NIH) 76-1165, Biological Safety Manual for Research Involving Oncogenic Viruses (This publication is available from the Superintendent of Documents, U.S.Government Printing Office, Washington, DC 20402)

Executive Order 12196 Safety and Health Programs for Federal Employees, 26 February 1980

Guide for Adult Immunizations, 2nd Ed., 1990(This publication is available from the American College of Physicians, Philadelphia, PA.)

Guide for Transportation of Hazardous Materials, Vol. 4(1) February 10, 1975 (This publication is available from the Office of Research Grants Inquiries, NIH, Department of Health and Human Services, 5333 Westbard Avenue, Bethesda, MD 20205.)

Guidelines for Laboratory Design, Health and Safety Considerations L. DiBerardinis, et. al., John Wiley and Sons,1987

Guidelines for Prevention of Herpesvirus Simiae (B Virus) Infection in Monkey Handlers, Mortality and Morbidity Weekly Report, Volume 36, Number 41; October 23, 1987, pages 680-689 (This publication is available from the Centers for Disease Control, Office of Biosafety, 1600 Clifton Road N.E., Atlanta, GA 30333. Telephone (404) 639–3883 or FTS 236–3883.)

HHS Publication No. (NIH) 88-8395 Biosafety in Microbiological and Biomedical Laboratories. (This publication is available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.)

Laboratory Safety for Arboviruses and Certain Other Viruses of Vertebrates, The American Journal of Tropical Medicine and Hygiene, 29:1359–1381, 1980.

Laboratory Safety: Principles and Practices.1986. Brinton M. Miller, Editor (This publication is available from the American Society for Microbiology Publications Office, 1913 I Street N.W., Washington, DC 20006.)

NIH publication 86–23, Guide for the Care and Use of Laboratory Animals (This publication is available from the Committee on Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission of Life Science, National Resource Council, Department of Health and Human Services, Public Health Service, Division of Research Resources, NIH, Bethesda, MD 20205.

NSF Standard #49
National Sanitation Foundation Standard Number 49, Class II (Laminar Flow) Biohazard Cabinetry

Packaging and Shipping of Biological Materials at The American Type Culture Collection (ATCC) (This publication is available from the ATCC, 12301 Parklawn Drive, Rockville, MD 20852. Telephone (301) 881–2600.)

Personnel and Product Protection: A Guide to Laboratory Equipment. 1983 (This publication is available from the Labconco Corporation, Kansas City, MO.)

Procedures for the Domestic Handling and Transport of Diagnostic Specimens and Etiologic Agents, National Committee for Clinical Laboratory Standards (NCCLS), (H5-A2), Second Edition. Vol. 5, No. 1 (This publication is available from the NCCLS, 771 East Lancaster Avenue, Villanova, PA 19085.)

Official Air Transport Restricted Articles Tariff 6-D (This publication is available from the Airline Tariff Publishing Co., Inc., 5724 Pulaski Road, Chicago, IL 60646, Telephone: (312) 478–0900.)

Technical Instructions for the Safe Transport of Dangerous Goods by Air (This publication is available from the International Civil Aviation Organization (ICAO) Intereg Group, 5724 Pulaski Road, Chicago, IL 60646, Tel. (312)478–0900.)

Section III
Prescribed Forms
This section contains no entries.

Section IV
Referenced Forms
This section contains no entries.

Appendix B
Laboratory safety inspection checklist

B–1. The checklist that follows is not an exhaustive list of the items to consider when inspecting facilities where etiologic agents are used. It does provide some basic guidelines to remind safety and nonsafety professionals of the things that need to be considered in the laboratories they manage. The checklist should be used as follows: All areas should be inspected using the general list in B–2. Certain items are optional, such as radiation safety. If no radioactive material is present in the room, then this would not be applicable. For BL–1 facilities the list in B–2 is adequate, while BL–2, BL–3, and BL–4 facilities must use the list in B–2 together with the appropriate list in B–3 to B–5.

B–2. Basic checklist

a. Housekeeping.

(1) Is the room free of clutter?

(2) Are all aisles from the work areas to the available exits maintained clear of obstructions?

(3) Are all safety equipment items unobstructed and ready for use?

(4) Is the room clean?

b. Fire safety.

(1) Is the fire extinguisher hung in its proper place, ready for use, and unobstructed?

(2) Are there excess flammables located outside National Fire Protection Association (NFPA) approved cabinetry?

(3) Are all Class IA flammables that are in breakable containers in pint or smaller containers?

(4) Are all Class IB flammables that are in breakable containers in liter or smaller containers?

c. Chemical safety.

(1) Are the chemicals stored with compatible materials?

(2) Have the chemical fume hoods been certified in the last 6 months?

(3) Are the eyewash and deluge shower unobstructed and ready for use?

(4) Is the eyewash and deluge shower tested regularly to document proper operation?

(5) Is the organic waste container maintained in a closed position?

(6) Are all reagents and solutions properly labeled?

(7) Is a spill kit within a reasonable distance from the work areas?

(8) Is appropriate protective clothing available for the chemical hazards present?

(9) Is there a written hazard communication program?

(10) Have the personnel in the laboratory been trained in the provisions and principles of the hazard communication program?

(11) Are MSDSs located where they are available to the laboratory workers?

(12) Is there a written chemical hygiene plan?

d. Radiation safety.

(1) Are the radioactive materials stored double contained?

(2) Is the containment for the radiation waste container adequate to preclude the spread of radiation?
(3) Are all containers appropriately labeled with radiation labels?
(4) Are all entrances to the room appropriately labeled?
e. Electrical safety.
(1) Are excess extension cords being utilized?
(2) Are there any frayed cords in the room?
(3) Are there any cords on the floor across normal traffic patterns in the room?
f. General laboratory safety.
(1) Are sharps discarded and destroyed in a safe manner?
(2) Are work surfaces decontaminated daily and after a spill?
(3) Is the appropriate attire worn by everyone in the room?
(4) Is there evidence that personnel eat, drink, smoke, or store food, drinks, or tobacco in the room?
(5) Was mouth pipetting observed?
(6) Are all gas cylinders secured and are all cylinders not in use capped?
(7) Are cylinders of oxidizers stored at least 20 feet from cylinders of flammable gases in the same room?
(8) Are the contents of the cylinders clearly labeled?
(9) Are the cylinders transported on appropriate dollies or hand trucks?
(10) Is there a written respiratory protection program where respirators are used?
g. Etiologic agents.
(1) Are all containers of etiologic agents appropriately labeled?
(a) Are freezers, refrigerators, and similar storage units labeled with the biohazard warning sign?
(b) Are the storage and shipping containers adequate and properly labeled?
(2) Have all personnel been adequately trained in general microbiological techniques?
(3) Are laboratory doors kept closed when experiments are in progress?
(4) Are all operations conducted over plastic backed absorbent paper or spill trays?

B–3. Biosafety level 2 supplemental checklist

a. Are all floor drains filled with water or suitable disinfectant?
b. Is the SOP for an etiologic agent spill signed by all personnel who work with etiologic agents in the room?
c. If biological safety cabinets are used, have they been certified within the last year?
d. Are the appropriate decontaminants available?
e. Are all entrances to the laboratory posted with—
(1) The appropriate special provisions for entry?
(2) The universal biohazard symbol?
(3) The name and telephone number of the laboratory director or other responsible person?
f. Is entry limited and restricted?
g. Are gloves being worn when handling infected animals or infectious or toxic materials?
h. Is eye and respiratory protection being worn in rooms where nonhuman primates are present?
i. If materials are being transported off-site for decontamination, is the containment adequate?

B–4. Biosafety level 3 supplemental checklist

a. Is laboratory clothing decontaminated before being sent to the laundry?
b. Are all windows and penetrations through the walls and ceilings sealed?
c. If biological safety cabinets are used, have they been certified within the last year?
d. Are the appropriate decontaminants available?
e. Are all entrances to the facility posted with—
(1) The appropriate special provisions for entry?
(2) The universal biohazard symbol?
(3) The name and telephone number of the laboratory director or other responsible person?
f. Is entry limited and restricted?
g. Are gloves being worn when handling infected animals or infectious or toxic materials?
h. Is eye and respiratory protection being worn in rooms where nonhuman primates are present?
i. Do the monitors indicate that the room is under negative pressure relative to all entrances?
j. Are all vacuum lines protected with HEPA filters and liquid disinfectant traps?
k. Is the autoclave being properly maintained and certified?
l. Is the foot, elbow, or automatic handwash sink operating properly?
m. Are all operations with etiologic agents being conducted inside biological safety cabinets or other approved engineering controls?
n. Are all infected animals housed using appropriate primary containment systems?
o. Do all personnel who enter rooms housing infected animals wear appropriate respiratory protection?
p. Do personnel who exit rooms having infected animals leave their protective clothing in the animal and laboratory rooms?
q. If a UV passbox is available, has its output been certified within the last 3 months?

B–5. Biosafety level 4 supplemental inspection checklist

a. Precautions for all areas.
(1) Are all penetrations through the walls and ceilings sealed?
(2) Are the appropriate decontaminants available and used properly?
(3) Are all entrances to the facility posted with—
(a) The appropriate special provisions for entry?
(b) The universal biohazard symbol?
(c) The name and telephone number of the laboratory director or other responsible person?
(4) Is access to the laboratory controlled strictly and documented?
(5) Do the monitors indicate that the room is under negative pressure relative to all entrances?
(6) Are all vacuum lines protected with HEPA filters and liquid disinfectant traps?
(7) Is the autoclave being properly maintained and certified?
(8) Is the foot, elbow, or automatic handwash sink operating properly?
(9) Do the self closing doors to the facility operate properly?
(10) Do personnel completely exchange street clothing for laboratory clothing before entry and shower upon exiting?
(11) Is the dunk tank disinfectant fresh and appropriate for the agents in use?
b. Suit areas.
(1) Are all operations with etiologic agents conducted in Class I or II biological safety cabinets?
(2) Do the procedures in place ensure that, as much as possible, the contamination remains inside the cabinets (such as ensuring that everything removed from within the cabinets, such as gloves being worn, instruments, glassware, or similar items), are decontaminated, or properly packaged first?
(3) Are the Class I or II cabinets in the facility certified every 6 months?
(4) Does the suit decontamination shower have adequate appropriate decontaminant available?
(5) Has the suit decontamination shower been used or tested in the last month?
(6) Is the ventilated suit air supply and emergency air supply adequate and working properly?
(7) Is the emergency alarm system working properly?
(8) Are all of the one-piece positive pressure suits available for use in serviceable condition?
(9) Are infected animals housed in appropriate primary containment systems?

(10) Is the static pressure in the suit area negative to all surrounding areas?

c. Nonsuit areas.

(1) Are all operations with etiologic agents conducted inside Class III biological safety cabinets?

(2) Were the Class III biological safety cabinets certified before personnel initiated the current operation?

(3) Are all infected animals housed in Class III cabinet containment caging systems?

Glossary

Section I
Abbreviations

AMC
U.S. Army Material Command

ANSI
American National Standards Institute

AR
Army Regulation

ARNG
Army National Guard

CFR
Code of Federal Regulations

DA
Department of the Army

DA Pam
Department of the Army Pamphlet

DNA
deoxyribonucleic acid

DOD
Department of Defense

DOT
Department of Transportation

EPA
Environmental Protection Agency

FDA
Food and Drug Administration

ICAO
International Civil Aviation Organization

min
minute

NEPA
National Environmental Policy Act

NIH
National Institute of Health

NRC
Nuclear Regulatory Commission

PPM
parts per million

RDTE
research, development, test, and evaluation

SOP
standing operating procedure

Section II
Terms

Approved respiratory protection
Equipment which is tested and listed as satisfactory according to standards established by a competent authority (such as NIOSH, Mine Safety and Health Administration (MSHA), or host country agency) to provide respiratory protection against the particular hazard for which it is designed. For military agent protection, DA and Department of Defense (DOD) are the approval authorities.(Approval authority may be specified by law.)

Biocontainment area
An area which meets the requirements for a BL–3 or BL–4 facility. The area may be an entire building or a single room within a building. See chapter 7 for details.

Biological safety cabinets
Engineering controls designed to enable laboratory workers to handle infectious etiologic agents and to provide primary containment of any resultant aerosol. There are three major classes of cabinets (I, II, and III) and several subclasses of class II cabinets. Each type of cabinet provides a different degree of protection to personnel and to the products handled inside them. The various classes of cabinets are described in detail in chapter 8.

Biosafety level 1
The facilities, equipment, and procedures suitable for work involving agents of no known or of minimal potential hazard to laboratory personnel and the environment.

Biosafety level 2
The facilities, equipment, and procedures applicable to clinical, diagnostic, or teaching laboratories, and suitable for work involving indigenous agents of moderate potential hazard to personnel and the environment. It differs from BL–1 in that—

a. Laboratory personnel have specific training in handling pathogenic agents.

b. The laboratory is directed by scientists with experience in the handling of specific agents.

c. Access to the laboratory is limited when work is being conducted.

d. Certain procedures in which infectious aerosols could be created are conducted in biological safety cabinets or other physical containment equipment.

Biosafety level 3
The facilities, equipment, and procedures applicable to clinical, diagnostic, research, or production facilities in which work is performed with indigenous or exotic agents where potential exists for infection by aerosol, and the disease may have serious or lethal consequences. It differs from BL–2 in that—

a. More extensive training in handling pathogenic and potentially lethal agents is necessary for laboratory personnel.

b. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets, other physical containment devices, or by personnel wearing appropriate personal protective clothing and devices.

c. The laboratory has special engineering and design features, including access zones, sealed penetrations, and directional airflow.

d. Any modification of BL–3 recommendations must be made only by the commander or director.

Biosafety level 4
The facilities, equipment, and procedures required for work with dangerous and exotic agents which pose a high individual risk of life threatening disease. It differs from BL–3 in that—

a. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents.

b. Laboratory personnel understand the primary and secondary containment functions of the standard and special practices, containment equipment, and laboratory design characteristics.

c. Access to the laboratory is strictly controlled by the institute director.

d. The facility is either in a separate building or in a controlled area within a building, completely isolated from all other areas of the building.

e. A specific facility operations manual is prepared or adopted.

f. Within work areas of the facility, all activities are confined to Class III biological safety cabinets or Class I or Class II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life support system.

g. The maximum containment laboratory has special engineering and design features to prevent microorganisms from being disseminated to the environment.

Building
A structure that contains the requisite components necessary to support a facility that is designed according to the required biosafety level. The building can contain one or more facilities conforming to one or more biosafety level.

Confirmed exposure
Any mishap with a BDP agent in which there was direct evidence of an actual exposure such as a measurable rise in antibody titer to the agent or a confirmed diagnosis of intoxication or disease.

Etiologic agents
Any viable microorganisms, or their toxin which causes or may cause human disease, including those agents listed in 42 CFR 72.3 of the Department of Health and Human Services regulations, and any agent of biological origin that poses a degree of hazard similar to those agents.

Facility
An area within a building that provides appropriate protective barriers for persons working in the facility and the environment external to the facility, and outside of the building.

HEPA filter
A filter which removes particulate matter down to submicron sized particles from the air passed through it with a minimum efficiency of 99.97 percent. While the filters remove particulate matter with great efficiency, vapors and gases (for example, from volatile chemicals) are passed through without restriction. HEPA filters are used as the primary means of removing infectious agents from air exhausted from engineering controls and facilities.

Human lethal dose
The estimated quantity of a toxin that is a minimum lethal dose for a 70 kilogram individual based upon published data or upon estimates extrapolated from animal toxicity data.

Institute director or commander
The institute director or commander of an Army activity conducting RDTE with BDP etiologic agents, or the equivalent, at a research organization under contract to the BDP.

Institution
An organization such as an Army RDTE activity (institute, agency, center, and so forth) or a contract organization such as a school of medicine, or research institute that conducts RDTE with BDP etiologic agents.

Laboratory
An individual room or rooms within a facility that provide space in which work with etiologic agents can be performed. It contains all of the appropriate engineering features and equipment required at a given biosafety level to protect personnel working in it and the environment external to the facility.

Large-scale operations
Research or production involving viable etiologic agents in quantities greater than 10 liters of culture.

Maximum containment area
An area which meets the requirements for a BL–4 facility. The area may be an entire building or a single room within the building. See chapter 7 for details.

Molded masks
Formed masks that fit snugly around the mouth and nose and are designed to protect against a nontoxic nuisance level of dusts and powders. These do not require approval by NIOSH or MSHA. Masks made of gauze do not qualify.

Potential accidental exposure
Any accident in which there was reason to believe that anyone working with a BDP agent may have been exposed to that agent, yet no measurable rise in antibody titer or diagnosis of intoxication or disease was made. However, the high probability existed for introduction of an agent through mucous membranes, respiratory tract, broken skin, or the circulatory system as a direct result of the accident, injury, or incident.

Resource Conservation Recovery Act of 1976 Listed Hazardous Waste
The waste materials listed by the Environmental Protection Agency under authority of the RCRA for which the agency regulates disposal. A description and listing of these wastes is located in 40 CFR part 261.

Suite
An area consisting of more than one room, designed to be a functional unit in which entire operations can be facilitated.Suites may contain a combination of laboratories or animal holding rooms and associated support areas within a facility that are designed to conform to a particular biosafety level. There may be one or more suites within a facility.

Toxin
Toxic material of etiologic origin that has been isolated from the parent organism. The publication "Bacterial Toxins:a Table of Lethal Amounts," (Gill, D.M. (1982)Microbiological Reviews, 46:86–94) contains a useful table of mammalian toxicities of numerous toxins.

Section III
Special Abbreviations and Terms

AIHA
American Industrial Hygiene Association

ASHRAE
American Society of Heating, Refrigerating, and Air Condition Engineers, Inc.

ATCC
American Type Culture Collection

BDP
Biological Defense Program

BL
biosafety level

CDC
Centers for Disease Control

EEE
Eastern equine encephalitis

EtO
ethylene oxide

fpm
feet per minute

HEPA
high efficiency particulate air

HHS
Health and Human Services

IATA
International Air Transport Association

IBC
Institutional Biosafety Committee

JE
Japanese Encephalitis

lfpm
linear feet per minute

LS
large-scale

m
meter

MSDS
Material Safety Data Sheets

MSHA
Mine Safety and Health Administration

NCCLS
National Committee for Clinical Laboratory Standards

NCI
National Cancer Institute

NFPA
National Fire Protection Association

NIOSH
National Institute for Occupational Safety and Health

NSF
National Sanitation Foundation

OSHA
Occupational Safety and Health Administration

pH
the negative logarithm of hydrogen ion concentration

PHS
Public Health Service

PPE
personal protective equipment

psi
pounds per square inch

RCRA Listed
Resource Conservation Recovery Act of 1976 Listed Hazardous Waste

RPO
Radiation Protection Officer

SALS
Subcommittee on Arbovirus Laboratory Safety

SAR
supplied air respirator

SCBA
self contained breathing apparatus

TD
to deliver

TLV
threshold limit value

USDA
U.S. Department of Agriculture

WEE
Western equine encephalitis

UV
ultraviolet

VEE
Venezuelan equine encephalitis

Index

This index is organized alphabetically by topic and by subtopic within a topic. Topics and subtopics are identified by paragraph number.

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